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Gamma-Glutamyl Transpeptidase: Redox Regulation and Drug Resistance

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Abstract

Expression of gamma-glutamyl transpeptidase (GGT) is essential to maintaining cysteine levels in the body. GGT is a cell surface enzyme that hydrolyzes the gamma-glutamyl bond of extracellular reduced and oxidized glutathione, initiating their cleavage into glutamate, cysteine (cystine) and glycine. GGT is normally expressed on the apical surface of ducts and glands, salvaging the amino acids from glutathione in the ductal fluids. GGT in tumors is expressed over the entire cell membrane and provides tumors with access to additional cysteine and cystine from reduced and oxidized glutathione in the blood and interstitial fluid. Cysteine is rate-limiting for glutathione synthesis in cells under oxidative stress. Induction of GGT is observed in tumors with elevated levels of intracellular glutathione. Studies in models of hepatocarcinogenesis show that GGT expression in foci of preneoplastic hepatocytes provides a selective advantage to the cells during tumor promotion with agents that deplete intracellular glutathione. Similarly, expression of GGT in tumors enables cells to maintain elevated levels of intracellular glutathione and to rapidly replenish glutathione during treatment with pro-oxidant anti-cancer therapy. In the clinic, expression of GGT in tumors is correlated with drug resistance. Inhibitors of GGT block GGT-positive tumors from accessing the cysteine in extracellular glutathione. They also inhibit GGT activity in the kidney, which results in excretion of GSH in the urine, and a rapid decrease in blood cysteine levels, leading to depletion of intracellular GSH in both GGT-positive and GGT-negative tumors. GGT inhibitors are being developed for clinical use to sensitize tumors to chemotherapy.

Keywords

Gamma-glutamyl transpeptidase; gamma glutamyl transferase; cysteine; cysteine; glutathione

INTRODUCTION

In 1985, gamma-glutamyl transpeptidase (GGT, a.k.a. gamma-glutamyl transferase) was first proposed to play a role in tumor formation (M. H. Hanigan & Pitot, 1985b). Preneoplastic liver foci in rats treated with chemical carcinogens were identified by their expression of GGT (Goldsworthy, Hanigan, & Pitot, 1986). We proposed that expression of

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GGT provided the cells within the foci a selective growth advantage during the promotion phase of carcinogenesis (M. H. Hanigan & Pitot, 1985b). This hypothesis was based on the observation that the treatment regimens which gave rise to GGT-positive liver foci all included promoting compounds that depleted glutathione (GSH). The hypothesis was that GGT, which is localized to the cell surface, cleaved extracellular GSH, thereby providing the cell with the amino acids necessary for intracellular GSH synthesis. GGT activity enabled the cells to maintain their intracellular GSH levels, thus resisting the toxicity of the promoting compounds and enabling them to respond to the proliferative signals triggered by the carcinogenic regimen. Now, decades later, there is a great deal of new information about the enzyme that supports this hypothesis. These data will be reviewed in this chapter. Further, studies from many laboratories have demonstrated that this same mechanism, through which GGT was proposed to contribute to the preneoplastic cell's resistance to the toxicity of promoting agents, also confers GGT-positive tumors with resistance to pro-oxidant cancer therapy. GGT enhances the tumor's access to cysteine, thereby increasing the intracellular GSH level. This enables the tumors to maintain their redox balance during the onslaught of reactive oxygen species (ROS) generated by the pro-oxidant therapies and to avoid death via the cell death pathways triggered by oxidative stress. Clinical studies have shown a correlation between GGT expression in human tumors and their resistance to therapy. Studies in cell culture and data from animal models have provided information on the signaling and regulatory pathways that underlie this correlation. To understand the relationship between GGT expression and drug resistance, it is necessary to first understand the role of GGT in normal physiology. This chapter will review the current information about GGT and its role in redox regulation, its expression in tumors, its induction by toxins including many of the most commonly used chemotherapy agents, and a strategy to use GGT inhibitors to overcome the resistance of both GGT-positive and GGT-negative tumors to pro-oxidant therapy.

EXPRESSION OF GGT AND DRUG RESISTANCE IN HUMAN TUMORS

Clinical studies show a strong correlation between expression of GGT in tumors and poor survival. Our study of 451 human tumors prior to treatment showed that GGT is induced during development of many tumors (M. H. Hanigan, Frierson, et al., 1999). Tumors derived from ductal epithelial cells that normally express GGT, were generally strongly GGT-positive (M. H. Hanigan & Frierson, 1996). These include liver, renal, prostatic, pancreatic, and breast carcinomas (Table I). Due to the ubiquitous expression of GGT in liver, renal, prostatic and pancreatic cancers, it is not possible to study the effect of GGT on the clinical outcome of these tumors. However, studies in breast, ovarian and other types of tumors demonstrated a positive correlation between GGT expression, tumor progression and poor overall survival of the patients. GGT is normally expressed in breast ductal epithelium and we found that all benign breast lesions were GGT-positive (Durham, Frierson, & Hanigan, 1997; M. H. Hanigan & Frierson, 1996). However, we observed that GGT expression was lost in some breast tumors (Durham, et al., 1997). Bard and colleagues reported that GGT-positive breast tumors were more likely to be estrogen-receptor negative tumors, which have a poor prognosis, and were more likely to have metastasized at the time of diagnosis, suggesting that expression of GGT provided a selective advantage to the tumor (Bard, Noel,

Chauvin, & Quash, 1986). GGT is also induced prior to therapy in some human tumors derived from tissues that do not normally express GGT, such as ovarian adenocarcinomas and soft tissue sarcomas. Among these tumors it has been observed that expression of GGT correlates with higher grade and drug resistant tumors. In a study of 634 ovarian cancer patients, Grimm and colleagues found that high pre-therapeutic expression of GGT served as a prognostic indicator of worse overall survival (Grimm et al., 2013). A smaller study of patients with Stage III and IV ovarian cancer found that GGT-positive and GGT-negative tumors had a similar response to initial therapy, but patients with GGT-positive tumors had a poorer 2 year survival rate than those with GGT-negative tumors (M. H. Hanigan, Frierson, & Taylor, 1998). In soft tissue sarcomas, GGT activity is higher in high-grade tumors and in metastases than in low-grade tumors (Hochwald, Rose, Brennan, & Burt, 1997). Induction of GGT has been observed in the drug resistant tumors that arise during treatment. In a set of ovarian cancer cell lines derived from a patient before and after the development of resistance to cisplatin, chlorambucil and 5-fluorouracil, drug-resistant cells exhibited a 6.5-fold induction of GGT activity (Lewis, Hayes, & Wolf, 1988).

As described in detail throughout this review, the mechanism underlying the correlation between expression of GGT and drug resistance is the ability of GGT to cleave extracellular GSH and thereby, provide cells with an additional source of cysteine with which to increase intracellular GSH levels. The cell lines derived before and after the development of drug resistance had elevated levels of both GGT and GSH (Lewis, et al., 1988). Induction of both intracellular GSH levels and GGT was also observed in human tumor cell lines selected in culture for resistance to chemotherapy (Godwin et al., 1992).

STRUCTURE OF GGT

In eukaryotes, GGT is a cell surface glycoprotein. It is anchored in the cell membrane via a single N-terminus transmembrane domain. All of the catalytic activity is within the extracellular domain of the protein (Ikeda, Fujii, Taniguchi, & Meister, 1995). Human GGT is synthesized as a 569 amino acid propeptide (Rajpert-De Meyts, Heisterkamp, & Groffen, 1988). The propeptide is enzymatically inactive, but is activated by autocleavage into two subunits (West et al., 2010; West et al., 2011). The autocleavage is dependent on an internal nucleophilic threonine (Thr 381), indicating that GGT is a member of the N-terminal nucleophilic (Ntn) hydrolase family, a group of enzymes with similar autocleavage and kinetic mechanisms but no sequence homology (Brannigan et al., 1995; Galperin & Koonin, 2012). N-glycosylation is essential for proper folding, autocleavage and activation of human GGT, although, the enzyme retains its activity when the glycans are removed from the mature, enzymatically active heterodimer (West et al., 2013; West, et al., 2011).

We recently solved the crystal structure of human GGT, the first crystal structure for any eukaryotic GGT (West, Chen, et al., 2013). The structure revealed a heterodimer with a stacked α - β - α core structure, a common element found in Ntn hydrolases (Galperin & Koonin, 2012). A surface representation of the enzyme reveals that the large subunit is wrapped around the small subunit (Fig. 1). Human GGT has seven N-glycosylation sites. The crystal structure showed that all seven sites are located on the external surface of the

protein (Fig. 1). Additional studies have shown site-specific and tissue-specific glycan compositional patterns on human GGT (West, et al., 2010).

The crystal structure of human GGT identified the substrate channel with the catalytic nucleophile (Thr-381) in the deepest part of the cleft. The relatively open active site in human GGT differs from the more restricted access found in most prokaryotic GGTs (Morrow, Williams, Sand, Boanca, & Barycki, 2007; Okada, Suzuki, Wada, Kumagai, & Fukuyama, 2006). In addition, we observed two different conformation for the side chain of the catalytic residue, Thr-381, suggesting that the side chain moves during substrate binding.

BIOCHEMISTRY OF GGT-CATALYZED REACTIONS

GGT is localized to the cell surface and only cleaves extracellular substrates. GSH and oxidized GSH (GSSG) are the most abundant physiological substrates, although GGT cleaves any gamma-glutamyl substrate including GSH S-conjugates (Wickham, West, Cook, & Hanigan, 2011). The substrate glutamate moiety must be unrestricted except for the gamma-glutamyl bond (Fig. 2). GGT will not cleave gamma-glutamyl bonds formed with glutamate that is within a peptide or with glutamate bound to any other compound.

The physiological reaction catalyzed by GGT is the hydrolysis of gamma-glutamyl bonds (PetitClerc, Shiele, Bagrel, Mahassen, & Siest, 1980) (Fig. 2). This is a multi-step reaction that proceeds by a mechanism common to Ntn hydrolases (Galperin & Koonin, 2012; Oinonen & Rouvinen, 2000). Thr-381 is the N-terminus residue of the small subunit and therefore, has a free amino group. As the substrate binds in the active site, the hydroxyl group on the side chain of Thr-381 is deprotonated by its free amino group (Castonguay et al., 2007). The side chain oxygen of Thr-381 initiates a nucleophilic attack on the carbonyl carbon of the gamma-glutamyl bond of the substrate. A transient, covalent acyl-bond is formed between the gamma-carbon of the gamma-glutamyl substrate and the hydroxyl (beta-oxygen) on the side chain of Thr-381 of GGT (Fig. 3) (Keillor, Castonguay, & Lherbet, 2005). This results in the release of all but the γ -glutamyl group of the substrate. Hydrolysis of the acyl bond is the rate-limiting step in the reaction and results in the release of glutamate. We have developed an assay that quantifies this activity at physiologic pH by measuring the release of glutamate (Wickham, West, et al., 2011). This assay can be used to evaluate the K_m for any gamma-glutamyl substrate and the velocity which GGT hydrolyzes the substrate. With GSH as the substrate, cysteinylglycine is the first product released and glutamate is the second product. The K_m for GSH is 11 μ M, consistent with the concentration of GSH in human serum and interstitial fluid (Wickham, West, et al., 2011). The K_m for GSSG is only slightly lower at 9 μ M, the rate of the reaction is the same for GSH and GSSG (Wickham, West, et al., 2011).

There was confusion for many years regarding the physiological role of GGT. GGT was discovered as a glutathionase and its hydrolysis of the gamma-glutamyl bond was first reported in 1948 (Binkley & Nakamura, 1948). But, several years later it was reported that in the presence of high concentrations of a dipeptide acceptor, GGT could catalyze a transpeptidation reaction, transferring the gamma-glutamyl group from the substrate to an acceptor, yielding a new gamma-glutamyl compound (Hanes, Hird, & Isherwood, 1952).

The enzyme, originally called glutathionase, was renamed gamma-glutamyl transpeptidase (Hanes, et al., 1952). Based on the transpeptidation reaction, GGT was proposed to be the central component of an amino acid transport system called the Gamma-Glutamyl Cycle (Meister, 1973; Orłowski & Meister, 1970). However, kinetic and functional studies have shown that the physiological function of GGT is that of a hydrolase rather than a transpeptidase (Curthoys & Hughey, 1979; Woodlock et al., 1990). Yet confusion persists in the literature. Adding to the confusion is the fact that the standard GGT biochemical assay measures the transpeptidation reaction (Fig. 3). The assay includes millimolar concentrations of γ -glutamyl-para-nitroanalide (GpNA) as a substrate, and 10 to 40 mM glycylglycine as an acceptor (Orłowski & Meister, 1963; Tateishi, Higashi, Nomura, Naruse, & Nakashima, 1976). Although this is not the physiological reaction, this transpeptidation appears to be uniquely catalyzed by GGT, and this colorimetric assay can be used to easily quantify GGT.

FUNCTION OF GGT

IN NORMAL TISSUES AND IN TUMORS

GGT is expressed on the luminal surface of excretive and absorptive cells that line glands and ducts throughout the body, with the highest level of GGT activity in the kidney (M. H. Hanigan & Frierson, 1996). The development of strains of GGT-knockout mice revealed the role of GGT in the distribution of cysteine throughout the body (Harding et al., 1997; Lieberman et al., 1996; Yamada, Tsuji, & Kunieda, 2013). GGT-knockout mice excrete 2500-fold more GSH in their urine than wild-type mice (Lieberman, et al., 1996). The glutathionuria is the result of the absence of GGT activity on the apical surface of the proximal tubules of the kidney. The cells lining the renal tubules are unable to take up GSH. In the GGT-knockout mice, GSH remains intact in the glomerular filtrate as it transits the kidney and the GSH is, thus, excreted in the urine. The continuous excretion of GSH results in a cysteine deficiency. The cysteine concentration in the plasma of GGT-knockout mice is only 20% of that seen in wild-type mice (Lieberman, et al., 1996). The mice fail to grow normally and die at approximately 10 weeks due to the cysteine deficiency. The GGT-knockout mice can be rescued by supplementing their drinking water with N-acetyl-cysteine (Lieberman, et al., 1996). Prior to death, the mice develop cataracts, have increased levels of oxidative DNA damage and are susceptible to oxygen-induced lung injury, all symptoms of reduced intracellular GSH and redox stress (Barrios et al., 2001; Chevez-Barrios, Wiseman, Rojas, Ou, & Lieberman, 2000; Rojas, Valverde, Kala, Kala, & Lieberman, 2000). Glutathionuria has also been reported in the few patients that have been identified with a GGT-deficiency (Wright, Stern, Ersser, & Patrick, 1980)(Schulman et al., 1975). Therefore, expression of GGT is essential to maintain cysteine homeostasis and protect tissues against oxidative stress.

Many tumors express GGT, but they are not polarized and, therefore, express GGT on their entire cell surface (M. H. Hanigan, Frierson, et al., 1999). Unlike normal cells in which GGT only has access to substrates in ductal fluids, the GGT on tumor cells can cleave GSH in interstitial fluid and blood. The expression of GGT provides tumor cells with an additional source of cysteine and cystine from the cleavage of extracellular GSH and GSSG.

This has been demonstrated by several investigators using cells transfected with GGT. We showed that Hepa 1–6 cells, a GGT-negative mouse liver tumor cell line, were unable to metabolize GSH in media nor were they able to take up GSH or GSH-derived cysteine (M. H. Hanigan, 1995). However, Hep 1–6 cells transfected with GGT rapidly metabolized ³⁵S-labelled GSH in the tissue culture media. Cysteinylglycine, a product of the GGT cleavage of GSH, was hydrolyzed by cell surface dipeptidases into cysteine and glycine and the GSH-derived ³⁵S-labelled cysteine was taken up into the cells (M. H. Hanigan, 1995). In other studies, GGT-positive NIH3T3 cells and GGT-negative controls grew at a similar rate in standard tissue culture media, which contains more than three times the concentration of cysteine and cystine present in interstitial fluid. However, when the cells were incubated in cysteine-free media containing GSH, the GGT-positive cells were able to cleave the extracellular GSH and use the cysteine for continued growth, while the GGT-negative controls died (M. H. Hanigan & Ricketts, 1993). Similar studies in a human B cell lymphoblastoid cell line transfected with GGT showed that the survival advantage provided by GGT for cells in cysteine-free medium was blocked by inhibition of cystine and cysteine uptake, further demonstrating that GGT released the cysteine from GSH providing cysteine to the cell (Karp, Shimooku, & Lipsky, 2001). Inhibition of GGT with acivicin was used to demonstrate the ability of GGT to provide rat colon cancer cells with GSH-derived cysteine and maintain their intracellular GSH levels when cultured in cysteine-free media (Huseby et al., 2003). Studies in which intracellular GSH was depleted with diethyl maleate showed that GGT-transfected NIH3T3 fibroblasts cleaved extracellular GSH and replenished intracellular GSH levels more rapidly than the GGT-negative controls (Rajpert-De Meyts et al., 1992). Access to additional cysteine becomes critical in resisting the toxicity of pro-oxidant therapy as described below.

OTHER GGT SUBSTRATES

The GGT-knockout mice have also been used to investigate the role of GGT in metabolizing other gamma-glutamyl substrates. GGT is an essential enzyme in the renal mercapturic acid pathway, a detoxification pathway that metabolizes GSH *S*-conjugates to cysteine *S*-conjugates for excretion (Cooper & Hanigan, 2010). There are, however, some drugs that are conjugated to GSH and are activated to nephrotoxins via the renal mercapturic acid pathway (Anders & Dekant, 1998). Studies in GGT-knockout mice and in culture have shown that the chemotherapy drug, cisplatin, is metabolized to a nephrotoxin through this pathway, and inhibition of GGT blocks the nephrotoxicity of this drug (M. H. Hanigan, Gallagher, Taylor, & Large, 1994; M. H. Hanigan et al., 2001; Townsend & Hanigan, 2002).

Stark and colleagues proposed that the GGT cleavage of GSH in the presence of transitional metals leads to oxidative damage of cell surface proteins and membrane lipids (Glass & Stark, 1997; Stark, Zeiger, & Pagano, 1993). The mechanism underlying their hypothesis is that the sulfur on the cysteine becomes increasingly reactive as GSH is cleaved to cysteinylglycine and then to cysteine by GGT and dipeptidase. Transition metals catalyze auto-oxidation of the sulfur via a Fenton reaction which results in the production of oxygen radicals. GGT has been shown to participate in generating oxidative damage in cell culture (Corti et al., 2009; Dominici et al., 1999; Stark et al., 1994). However, the reactions are unlikely to occur under physiological conditions due to limited availability of the reactants

in extracellular fluids. The exception may be reperfusion injury, where inhibition of GGT in rats has been shown to be protective (Yamamoto et al., 2011). In contrast, mouse studies show that lack of GGT activity leads to oxidative damage due to a reduced supply of cysteine to the tissues (Rojas, et al., 2000).

OTHER GGT GENES

In the human genome, there is a family of GGT genes (Heisterkamp, Groffen, Warburton, & Sneddon, 2008). GGT1, which is generally referred to simply as GGT, is located at Chromosome 22q11 (Figlewicz et al., 1993; Morris et al., 1993). This region of Chromosome 22 has undergone multiple duplications and rearrangements. Most of the human GGT genes contain coding regions for only fragments of the GGT1 protein. Courtay and colleagues found mRNA expressed from four human GGT genes, later identified as *GGTLC2*, *GGTLC3*, *GGTLC4P* and *GGTLC5P* all of which encode only the light chain of GGT and therefore would not have enzymatic activity since both subunits are needed for activity (Courtay, Heisterkamp, Siest, & Groffen, 1994; Heisterkamp, et al., 2008). However, *GGT2*, a gene that is located in this region of chromosome 22 and is closely related to *GGT1*, encodes a full length protein (Heisterkamp, et al., 2008). *GGT2* is likely a duplication of the *GGT1* gene that occurred late in evolution as *GGT2* is present in the human genome, but is not present in any other species including other primates (Heisterkamp, et al., 2008). There is 97% nucleotide identity between *GGT1* and *GGT2*, and 94% amino acid identity. *GGT2* mRNA has been detected and this has led to the assumption that *GGT2* encodes an enzymatically active protein (Auman et al., 2008; Moon et al., 2012). However, we have recently shown that GGT2 propeptides encoded by all three isoforms of GGT2 failed to autocleave, were enzymatically inactive, did not localize to the plasma membrane, and were rapidly degraded within the cell (West, Wickham, Parks, Sherry, & Hanigan, 2013).

The only GGT gene in addition to *GGT1* that has been shown to encode a protein with enzymatic activity is *GGT5*, which has been identified in humans and rodents (Carter, Shi, Barrios, & Lieberman, 1998; Heisterkamp, Rajpert-De Meyts, Uribe, Forman, & Groffen, 1991). *GGT5* was originally identified in mice based on its ability to cleave the GSH S-conjugate leukotriene C₄ to leukotriene D₄ and is implicated in inflammation (Z. Z. Shi, Han, Habib, Matzuk, & Lieberman, 2001). Mouse GGT5 does not cleave GSH (Z. Z. Shi, et al., 2001). The human *GGT5* gene has 40% amino acid identity to *GGT1*, but the second order rate kinetics show that human GGT5 has less than 4% of the activity of GGT1 in hydrolyzing GSH, GSSG and leukotriene C₄ (Heisterkamp, et al., 1991; Wickham, West, et al., 2011). There is no published information on GGT5 expression in tumors. Therefore, this chapter will focus exclusively on GGT1, which will be referred to as GGT.

GSH AND CYSTEINE IN REDOX REGULATION

GSH AND INTRACELLULAR REDOX REGULATION

GSH is the most abundant non-protein thiol in mammalian systems and the major redox buffer in the cell (Moriarty-Craige & Jones, 2004). GSH protects cellular components from oxidative damage of reactive oxygen species (ROS), such as hydrogen peroxide and organic

peroxides via GSH peroxidases and also detoxifies the electrophilic metabolites of toxins, including chemotherapy drugs (Moriarty-Craige & Jones, 2004) (Fig. 4). The ratio of oxidized GSH (GSSG) to 2GSH can be used as a measure of the intracellular redox environment (Schafer & Buettner, 2001).

Pro-oxidant therapies are defined as those that stress the redox balance in the cell towards a more positive (oxidized) state. These include many of the most commonly used anti-cancer treatments including platinum-based compounds, alkylating agents, anthracyclins and radiation (Mistry & Harrap, 1991). The GSH levels are often elevated in tumor cells prior to treatment because the high metabolic rate in tumors results in enhanced ROS production, which stresses their redox balance and induces GSH synthesis (Balendiran, Dabur, & Fraser, 2004; Cairns, Harris, & Mak, 2011; Chaiswing, Zhong, & Oberley, 2011; Kansanen, Kuosmanen, Leinonen, & Levonen, 2013; Pani, Galeotti, & Chiarugi, 2010). Many studies have shown a strong correlation between elevated levels of intracellular GSH and resistance to pro-oxidant chemotherapy (Balendiran, et al., 2004; Estrela, Ortega, & Obrador, 2006) (Newkirk K 40:75–80, 1997).

INCREASED REQUIREMENT FOR GSH FOR TUMORS AND CELLS UNDER REDOX STRESS

During redox stress there are multiple pathways by which toxins, including many chemotherapy drugs, deplete intracellular GSH (Fig. 4). GSH *S*-transferases catalyze the conjugation of GSH to electrophilic drug metabolites, which prevents the drugs from binding to DNA and other nucleophilic cellular components (Tew & Townsend, 2012). These GSH-conjugates are transported out of the cell by the multidrug resistance protein (MRP) transporters (Bachhawat, Thakur, Kaur, & Zulkifli, 2013; Cole & Deeley, 2006). In addition, GSH is oxidized to GSSG which is excreted by the MRP transporters during times of high oxidative stress to prevent its accumulation (Cole & Deeley, 2006). During redox stress intracellular GSH levels are further depleted as cysteine residues in some proteins undergo, *S*-glutathionylation, a process that can alter protein structure and function (Xiong, Uys, Tew, & Townsend, 2011).

Cells contain both redox sensing and redox-signaling systems, which regulate differentiation, proliferation and cell death (Chaudhari, Ye, & Jang, 2012; Go & Jones, 2013). As pro-oxidant drugs deplete reduced GSH from the cell, the redox environment becomes increasingly oxidized, which triggers pro-apoptotic signaling pathways and other death pathways (Circu & Aw, 2010; Jones, 2010; Ortega, Salvador, & Estrela, 2011). *S*-glutathionylation is also a consequence of oxidative stress and *S*-glutathionylation of substrates such as protein disulfide isomerase also triggers cell death through activation of the unfolded protein response and disruption of other cell signaling pathways (Xiong, et al., 2011). The drug-resistant cells with increased GSH synthesis are able to maintain intracellular GSH and avoid activation of the cell death pathways.

REPLENISHMENT OF GSH IS DEPENDENT ON CYSTEINE AND CYSTINE UPTAKE

Most normal cells and tumors cannot take up intact GSH. Intracellular levels of GSH are normally maintained by feedback regulation of the rate-limiting enzyme in GSH synthesis,

gamma-glutamyl cysteine synthetase (Meister, 1991). The cysteine and cystine concentrations in the serum and interstitial fluid are sufficient to enable cells to maintain intracellular GSH levels. However, under redox stress, increased amounts of GSH are synthesized and cysteine becomes rate-limiting for GSH synthesis (Ortega, et al., 2011). Tumors are, therefore, dependent on enhanced uptake of cysteine and cystine to maintain their intracellular GSH levels (Fig. 4) (Ishimoto et al., 2011). Cysteine is taken up into the cells via the alanine, serine, cysteine transporters (ASCTs) (Scopelliti, Ryan, & Vandenberg, 2013). Cystine, the oxidized form of cysteine, is present in human serum and interstitial fluid at 66 μ M, five times the concentration of cysteine and is a major source of cysteine for the cell (Blanco et al., 2007; Davis, Quinlivan, Stacpoole, & Gregory, 2006). Cystine is transported into the cell via the x_c^- cystine/glutamate antiporter (Davis, et al., 2006; Lewerenz et al., 2013).

Tumors have a high requirement for cysteine in order to maintain their intracellular GSH levels. Many tumors have enhanced uptake of cystine. xCT is the light chain of the x_c^- cystine/glutamate antiporter that transports cystine into the cell (Lewerenz, et al., 2013). Overexpression of xCT is commonly observed in drug-resistant tumors and is a marker for poor survival in patients (Kinoshita et al., 2013; Takeuchi et al., 2013). Blocking cystine uptake sensitizes these tumor cells to therapy (Doxsee et al., 2007; Pham et al., 2010; Vene et al., 2011). xCT overexpression has been identified in tumor stem cells. Epithelial tumor stem cells express the epithelial splicing regulator protein 1 (ESRP-1), a physiological regulator that maintains pluripotency in normal mouse and human epithelial stem cells (Fagoonee et al., 2013). Co-expression of ESRP-1 and CD44, a cell surface marker used to identify cancer stem cells, results in production of alternatively spliced variants of CD44 (Biddle, Gammon, Fazil, & Mackenzie, 2013; Warzecha, Shen, Xing, & Carstens, 2009). One of the CD44 variants, CD44v8-10, produces a highly metastatic and drug resistant population of tumor cells (Nagano, Okazaki, & Saya, 2013). The mechanism by which CD44v8-10 affects metastasis and drug resistance is by stabilizing xCT resulting in increased cystine uptake (Ishimoto, et al., 2011; Lewerenz, et al., 2013). Blocking cystine uptake negates the metastatic potential and drug resistance of CD44v8-10 expressing cells (Biddle, et al., 2013; Ishimoto, et al., 2011; Yoshikawa et al., 2013). There are no studies on the co-regulation of cystine transport and expression of GGT. Both can increase the supply of cysteine to the cell. They could work in tandem, with GGT initiating the release of cystine from extracellular GSSG, providing more cystine for uptake into the cell.

THE ROLE OF GGT IN ENHANCING CYSTEINE AVAILABILITY AND DRUG RESISTANCE

A dramatic shift in the availability of cysteine occurs when GGT-positive cells are depolarized or GGT is induced in GGT-negative cells. Under these conditions the GGT is no longer restricted to substrates present in the fluids within ducts and glands as it is in normal tissues, but now can cleave both oxidized and reduced GSH in interstitial fluid, providing the cell access to the cystine and cysteine therein. Cameron and colleagues reported that in rats with neoplastic liver nodules and carcinomas, GSH is excreted by the normal hepatocytes, but up to 80% of the GSH produced in the liver was cleaved by the GGT-

positive nodules and tumors (Cameron, Armstrong, Gunsekara, Varghese, & Speisky, 1991). When GGT was inhibited, the GSH in the venous fluid increased five-fold (Cameron, et al., 1991). The use of extracellular GSH by GGT-positive tumors *in vivo* was also demonstrated by measuring the GSH and cysteine concentrations in arterial and venous blood flowing through GGT-positive tumors implanted in the ovary (Hochwald, Harrison, Rose, Anderson, & Burt, 1996). A single pass of the blood through the tumor resulted in a 69% decrease in the serum GSH concentration, a significantly higher utilization rate than that observed in the systemic circulation. Administration of a GGT inhibitor blocked GSH degradation.

During the onslaught of toxins that deplete GSH including many chemotherapy drugs, expression of GGT provides cells with additional cysteine that is essential to overcoming the toxicity of the drug. In addition, the elevated levels of intracellular GSH maintains the redox status within the cells, enabling them to respond to the proliferative and differentiation signals present in the tissue following injury from the toxin. This was elegantly demonstrated in a study of coumarin-induced Clara cell toxicity in the lung (Vassallo et al., 2010). Clara cells normally express GGT. A single treatment with coumarin killed the Clara cells in the bronchiolar epithelium of both wild-type mice and GGT-knockout mice within 24 hours. However, during a twelve day period with repeated coumarin dosing, the Clara cells in the bronchial epithelium of the wild-type mice regenerated, whereas those in the GGT-knockout mice did not. An important component of this experiment was the fact that the GGT-knockout mice were fed NAC in their drinking water throughout the study, they had normal levels of cysteine in their serum, they were not cysteine-deficient. The GSH concentration in the lungs of the untreated controls was the same for the wild-type and GGT-knockout mice. In mice, Clara cells metabolize coumarin to an epoxide which is detoxified by conjugation to GSH (Vassallo, Hicks, Born, & Daston, 2004). GSH is rapidly depleted from the lung during metabolism of epoxides to GSH conjugates (Warren, Brown, & Buckpitt, 1982). The Clara cells that grew out in the wild-type mice had 13-fold higher GGT activity and 3.3 times the intracellular GSH concentration compared to the Clara cells in untreated controls. In GGT knockout mice the cells were unable to sustain adequate concentrations of GSH and could not regenerate.

Availability of cysteine can limit the growth of rapidly dividing tumors in mice, while expression of GGT can provide additional cysteine for an enhanced growth rate. Two GGT-negative skin cell lines which produce papillomas in nude mice, were transfected with GGT. When injected into nude mice, the GGT-positive clones grew more rapidly and resulted in more malignant tumors than the vector-transfected controls (Slaga, Budunova, Gimenez-Conti, & Aldaz, 1996). Similarly, the human PC3 tumor cell line transfected with GGT grew more rapidly in mice than its GGT-negative vector-transfected control (M. H. Hanigan, Gallagher, Townsend, & Gabarra, 1999). Generally rapidly growing tumors are more sensitive to alkylating agents; however, expression of GGT in PC3-tumors and access to additional cysteine not only increased the growth rate but also increased their resistance to chemotherapy (M. H. Hanigan, Gallagher, et al., 1999).

GGT is induced in mouse cells selected in culture for resistance alkylating agents. Ahmad and colleagues selected murine L1210 leukemia cells for resistance to L-phenylalanine mustard (L-PAM) and found that the drug resistant line had three-fold more intracellular

GSH and three-fold higher GGT activity (Ahmad, Okine, Wood, Aljian, & Vistica, 1987). The drug resistant cells metabolized more extracellular GSH and took up more GSH derived ^{35}S -cysteine. The authors concluded that the increased level of GGT in the drug resistant cells provided the cells with more cysteine with which to maintain their elevated intracellular GSH concentration and resist to toxicity of the alkylating agent.

GGT and other GSH related enzymes are induced in human cells selected in culture for resistance to pro-oxidant chemotherapeutic drugs. Selection of the human ovarian tumor cell lines, A2780 and A1847, for resistance to cisplatin yielded highly resistant clones that had 13- to 50-fold increased levels of intracellular GSH, enhanced expression of gamma-glutamyl cysteine synthetase and a 15- to 40-fold increase in GGT mRNA (Godwin, et al., 1992). Other investigators showed that the GGT mRNA and GGT activity in human ovarian cell lines increased within 48 hours of exposure to cisplatin (Oguchi et al., 1994).

Tissue culture studies on the role of GGT in cells under oxidative stress can be misleading if the cysteine levels in the media are not adjusted to physiological levels. The concentration of cysteine and cystine in standard tissue culture media ranges from 200 μM cystine (equivalent to 400 μM cysteine) in DMEM and RPMI-1640 to 990 μM cysteine in L-15 medium. In contrast, the concentration of cysteine and cystine in serum and interstitial fluids in humans are 12 μM and 66 μM respectively (Blanco, et al., 2007). Most tissue culture media does not contain GSH with the exception of RPMI-1640 which is formulated with 3.2 μM GSH. As described above, GGT-positive PC3 cells grew faster in mice than their GGT-negative controls due to their additional supply of cysteine, but when these cells were cultured in RPMI-1640 media containing 200 μM cystine, both cell lines grew at the same rate (M. H. Hanigan, Gallagher, et al., 1999). However, studies of cell growth in tissue culture have shown that media with lower levels of cysteine/cystine can be used to demonstrate the effect of GGT activity on increasing the cysteine supply to the cells. Incubation of Hep1-6 cells in media with various concentrations of cysteine showed that in media containing less than 80 μM cysteine, the GGT-positive cells grew more rapidly than the GGT-negative cells (M. H. Hanigan, 1995). Analysis of media that was on the cells for 24 hours showed that GSH was being secreted into the media by these liver-derived cells. GSH accumulated in the media on the GGT-negative cells, but could not be detected in the media on the GGT-positive cells because it was being degraded by the GGT. Salvaging the cysteine from the secreted GSH provided the GGT-positive cells with access to more cysteine and allowed for increased growth under conditions of limiting cysteine (M. H. Hanigan, 1995). A potential complication that can arise while investigating the effect of GGT or extracellular GSH in cell culture studies is the presence of GGT activity in some lots of commercial bovine serum (M. H. Hanigan, Brown, & Ricketts, 1993). It is essential that investigators planning these types of studies screen aliquots of the serum for GGT activity prior to purchasing it.

Studies done in tissue culture investigating the effect of transfection of GGT on drug sensitivity generally show no effect when the studies are done in complete media because there is no advantage to accessing additional cysteine when the cells are cultured in the very high level of cysteine present in tissue culture media (Bailey, Gipp, & Mulcahy, 1994). Some studies have reported a protective effect of GGT expression against the toxicity of

chemotherapy in human tumor cells cultured in complete media, likely due to the ability of the GGT-positive cells to cleave the GSH and GSSG they have excreted into the media. The toxicity of cisplatin and doxorubicin towards HepG2 cells was increased when GGT was inhibited by acivicin, but the effect was very modest compared to the potentiation of cytotoxicity observed in cells cultured in physiological concentrations of cysteine (Kwiecien, Rokita, Lorenc-Koci, Sokolowska, & Wlodek, 2007).

The protective effect of GGT against toxicity can be demonstrated in cells grown in standard media when the cells are under extreme oxidative stress. Shi and colleagues used GGT-transfected NIH3T3 cells to investigate the role of GGT in cells under oxidative stress (M. Shi, Gozal, Choy, & Forman, 1993). The GGT-positive and control GGT-negative cells were treated with 2,3-dimethoxy-1,4-naphthoquinone (DMNQ), which generates H₂O₂ intracellularly through redox cycling and caused a dose-dependent decrease in intracellular GSH in both cell lines. Addition of GSH to the media, resulted in maintenance of intracellular GSH and resistance to DMNQ-induced toxicity in the GGT-positive cells, but not the control cells. Treatment of the GGT-positive cells with an inhibitor of GGT blocked the protective effect of the extracellular GSH. This study demonstrates that in cells under oxidative stress, expression of GGT provided cells with access to an additional source of cysteine that helped maintain intracellular GSH and increased resistance to the toxicity of the drug.

REDOX REGULATION OF GGT

Expression of GGT and its role in redox regulation has been studied extensively in rats, mice and humans. However, there are differences in the expression and regulation of GGT among these species that are often not recognized, but provide insight into GGT regulation and function. Therefore, we will consider the data from each species separately.

REDOX REGULATION OF GGT EXPRESSION IN RATS

In the rat, GGT is a single copy gene and its expression is regulated by five tandemly arranged promoters that give rise to seven different transcripts all of which encode the same protein, but have distinct 5'-untranslated regions (Chikhi, Holic, Guellaen, & Laperche, 1999). The promoters are tissue specific and AP-1, AP-2 and NFκB binding sites have been identified in promoter region (Chikhi, et al., 1999). Few studies have been done in the rat *in vivo* to identify pathways that regulate GGT expression, although Yang and colleagues reported that, electroporation of a dominant negative ETV5 into the cells in an isolated inner segment of the epididymis resulted in a decreased expression of GGT mRNA IV (Yang, Fox, Kirby, Troan, & Hinton, 2006).

In vivo studies in rats demonstrated that oxidative stress in the lungs caused by hyperoxia, ozone, or nitrogen dioxide resulted in induction of GGT activity (Knickelbein et al., 1996; Takahashi, Oakes, et al., 1997; Takahashi, Takahashi, et al., 1997). Using primary isolated rat lung macrophages, Forman and Skelton showed that extracellular GSH and GGT were protective against hyperoxia-induced toxicity and inhibitors of GGT blocked the protective effect of extracellular GSH (Forman & Skelton, 1990).

Induction of GGT in rat liver has been studied extensively following treatment with toxins and hepatocarcinogens. In toxicology studies, GGT is induced in specific zones within the liver, dependent on the site of metabolism of the administered compound. For example, in rats fed ethoxyquin, GGT was induced in the periportal hepatocytes (Mandel et al., 1987). Experimental protocols for inducing hepatocellular carcinomas in rats consist of a single treatment with a mutagen followed by repeated doses of a promoting agent (Goldsworthy, et al., 1986). During the promotion phase, distinct foci of hepatocytes with altered levels of several enzymes can be identified. The most commonly used marker for identification of these enzyme-altered foci is the induction of GGT. We showed that the GGT-positive hepatocytes from carcinogen-treated rats have reduced levels of cytochrome P450 enzymes associated with metabolic activation of liver carcinogens (M. H. Hanigan & Pitot, 1985a). Stenius and colleague showed that these GGT-positive hepatocytes were resistant to GSH depletion and hydroquinone- or menadione-induced oxidative stress (Stenius, Rubin, Gullberg, & Hogberg, 1990). The GGT-positive hepatocytes are not polarized and GGT is expressed on their entire cell surface enabling them to use the GSH in the serum and interstitial fluid as an additional source of cysteine to increase their intracellular GSH levels. The GGT-positive hepatocytes have a selective advantage during the promotion phase of carcinogenesis as the liver is exposed repeatedly to promoting agents such as phenobarbital which induces oxidative stress and depletes intracellular GSH (Sies, Bartoli, Burk, & Waydhas, 1978). It is from within these enzyme-altered foci that the tumors arise. In protocols that use peroxisome proliferators as promoting agents, the GSH levels in the liver are not depleted and induction of GGT would not provide any selective advantage to the cells. The enzyme-altered foci and liver tumors in peroxisome-treated rats are GGT-negative (Rao, Lalwani, Scarpelli, & Reddy, 1982). These studies demonstrate a strong association between oxidative stress, depletion of GSH, induction of GGT and the selective outgrowth of GGT-positive hepatocytes in the rat (M. H. Hanigan & Pitot, 1985b).

Despite the induction of GGT in preneoplastic liver lesions by many different carcinogen treatments, it has not been possible to study this induction in primary culture. When rat hepatocytes are isolated and put in cell culture their GGT activity increases dramatically even in the absence of any carcinogen treatment. We found that GGT activity in rat hepatocytes increased 20-fold during the first 7 days in culture (Gallagher, Rudolph, Hinton, & Hanigan, 1998). Only GGT mRNA_{III} is induced indicating that promoter III is responsible for the induction (Gallagher, et al., 1998). GGT mRNA_{III} is the same GGT mRNA induced in rat liver tumors, and it contains the antioxidant response element consensus sequence and binding sites for NF-1 and NFY transcription factors (Brouillet et al., 1994). The oxygen levels in culture are much higher than the oxygen levels that hepatocytes are exposed to *in vivo*. These data suggest that the hyperoxia induces oxidative stress which triggers the induction of GGT. Interestingly, as the GGT levels in the hepatocytes increase the cystathionase activity decreases (Meredith, 1987). Cystathionase enables the cells to synthesize cysteine from methionine and a lack of cystathionase would increase their requirement for extracellular sources of cysteine in order to maintain intracellular GSH levels.

Redox regulation of GGT in rat tissues other than hepatocytes have been studied in culture. A time- and dose-dependent increase in GGT mRNA and GGT activity was reported in CC531 rat colon carcinoma cells exposed to ionizing radiation, which caused the formation of reactive oxygen and nitrogen species (Pankiv, Moller, Bjorkoy, Moens, & Huseby, 2006). In the same cell line, menadione treatment induced both ROS and GGT activity (Mikkelsen, Mortensen, Laperche, & Huseby, 2002). In a subsequent study, the authors reported that ROS induced GGT involved activation of Ras and was mediated through an AKT, p38 MAPK and MEK1 dependent pathway (Pandur, Pankiv, Johannessen, Moens, & Huseby, 2007).

REDOX REGULATION OF GGT EXPRESSION IN MICE

In the mouse, GGT is a single copy gene that can be transcribed from seven different promoters which give rise to distinct mRNAs that encode the same protein but differ in their 5' untranslated region (Lieberman et al., 1995). Induction of GGT is tissue-specific and murine GGT mRNA has been shown to undergo tissue-specific and developmentally regulated alternative splicing which gives rise to both active and enzymatically inactive GGT proteins (Chikhi, et al., 1999) (Joyce-Brady, Jean, & Hughey, 2001). Binding sites for AP-1 as well as the redox sensitive transcription factors AP-2 and nuclear factor κ B (NF κ B) have been identified in the murine promoters (Chikhi, et al., 1999).

Expression of GGT in response to oxidative stress has been observed in several tissues in the mouse. Using mouse embryo fibroblasts (MEF) from Nrf2-deficient mice and wild type controls, DeNicola and colleagues showed that expression of the oncogenic K-ras in wild type MEFs resulted in an induction of GGT mRNA (DeNicola et al., 2011). In this study, expression of K-ras and Myc oncogenes increased the transcription of Nrf2 and induced a series of genes including GGT, all of which are important in the cellular response to ROS (DeNicola, et al., 2011). In Nrf2 deficient cells GGT mRNA was reduced and was not altered by expression of the oncogenic K-ras indicating that K-ras was signaling through Nrf2 to induce GGT mRNA. This was confirmed in Nrf2 knockdown studies. GGT-deficient mice develop oxidant stress in the lung even under conditions of normoxia suggesting that GGT protects the lung from oxidative stress (Jean et al., 2002).

Induction of GGT is a commonly used marker for preneoplastic liver lesions in rats, but is rarely used in mice. Many strains of mice develop preneoplastic lesions and liver tumors spontaneously with age. The endogenous promoting agent for these tumors is unknown and GGT is not expressed in the preneoplastic lesions or tumors (Goldsworthy, et al., 1986). However, GGT is induced in these lesions when they are subjected to oxidative stress. Administration of phenobarbital to aged C3HfB/HeN mice for 5 to 7 weeks resulted in an induction of GGT in the tumors and in centrilobular hepatocytes, which was reversible when the phenobarbital was withdrawn (Williams, Ohmori, Katayama, & Rice, 1980). Phenobarbital induces oxidative stress, depletes intracellular GSH and is a commonly used promoting agent in rat hepatocarcinogenesis protocols that induce GGT-positive preneoplastic lesions and tumors (Goldsworthy, et al., 1986; Sies, et al., 1978). We found that different promoting agents used in initiation/promotion protocols in mice gave rise to preneoplastic foci that differed in their enzyme profile (M. H. Hanigan, Winkler, &

Drinkwater, 1993). GGT-positive foci were present in mice treated with *ortho*-azoaminotoluene or safrole as promoting agents. Both *ortho*-azoaminotoluene and safrole require metabolic activation in the liver to mutagenic compounds which cause oxidative stress (Nakagawa, Suzuki, Nakajima, Ishii, & Ogata, 2009). The GGT-positive foci were independent from the glucose-6-phosphatase deficient foci present in the same livers which were presumably being stimulated by endogenous promoters in the mice. Roomi and colleagues fed C3H mice griseofulvin, a hepatocarcinogen with both initiating and promoting activity (Roomi et al., 2006). The preneoplastic foci were GGT-positive and expressed glutathione *S*-transferase mμ. When the mice were injected with a hepatotoxic dose of thioacetamide the GGT-positive hepatocytes began to proliferate while necrosis was observed in the surrounding liver. Thioacetamide is metabolized in mouse liver by CYP2E and increases oxidative stress (Kang et al., 2008). The authors concluded that the GGT-positive, glutathione *S*-transferase mμ-positive preneoplastic foci had a survival advantage over the normal hepatocytes and were able to respond to the proliferation stimulus of the compensatory hyperplasia following the hepatotoxicity (Roomi, et al., 2006).

There is species specificity to the induction of GGT in primary cultures of hepatocytes. The concentration of GSH in the livers of rats and mice is similar (Vina, Perez, Furukawa, Palacin, & Vina, 1989). However, GGT is rapidly induced in rat hepatocytes when they are placed in culture, but there is no induction of GGT in mouse hepatocytes (Gallagher, et al., 1998). This difference between rat and mouse hepatocytes is only one of several differences between the hepatocytes of these two species with regard to GGT and other enzymes involved in GSH metabolism. Rats are very sensitive to the hepatotoxicity of aflatoxin B₁ while mice are resistant. Primary cultures of hepatocytes from these two species show this same difference in susceptibility to aflatoxin B₁ toxicity (H. M. Hanigan & Laishes, 1984). The resistance of the mouse hepatocytes has been shown to be the result of the constitutively high level of expression of an alpha class GSH *S*-transferase that inactivates the electrophilic metabolite of aflatoxin B₁ that is formed in the liver of both rats and mice (Hayes, Judah, Neal, & Nguyen, 1992). Hepatocytes can lose their ability to synthesize cysteine during transformation as shown with the mouse derived liver tumor cell line, Hep1-6, which is unable to survive in cysteine-free media as described in the GGT-transfection studies above (M. H. Hanigan, 1995).

REDOX REGULATION OF GGT EXPRESSION IN HUMANS

The transcriptional regulation of GGT in humans is tissue-specific with multiple mRNAs encoding the same open reading frame but differing in their 5' untranslated region (Daubeuf et al., 2001; Visvikis et al., 2001). The human GGT promoter contains binding sites for AP1, AP2, CREB, GRE, NF-κB and two Sp1 binding sites (Visvikis, et al., 2001)(Reuter et al., 2009). The 5'untranslated region of human GGT also contains multiple steroid modulatory elements (Diederich, Wellman, Visvikis, Puga, & Siest, 1993). Modulation of GGT expression by ras was demonstrated with HT29 cells and DLD-1 cells, two human colon carcinoma cells lines (Pankiv, et al., 2006). When HT29 cells, which express wildtype c-Ha-ras, were stably transfected with activated ras, the level of GGT protein increased and GGT activity increased approximately 2-fold compared to vector transfected controls. Conversely, DLD-1 cells, which express activated Ras (Ki-ras mutation) were transfected with the

dominant negative RasN17 mutant, their level of GGT expression decreased and their GGT activity was reduced to 50%.

Induction of GGT through redox signaling pathways has been demonstrated in human cell lines. Ravuri and colleagues reported that treatment of HT-29 cells, a human colon carcinoma cell line, with phorbol 12-myristate-13-acetate (PMA) activated the NADPH oxidase (NOX) system, increased intracellular ROS levels, induced GGT mRNA, increased GGT protein expression and elevated GGT activity (Ravuri, Svineng, Pankiv, & Huseby, 2011). In the same study, inhibition of NOX activity with apocynin down regulated GGT mRNA, protein levels and GGT activity. Tumor necrosis factor alpha (TNF α) was shown to induce GGT expression through the NF- κ B signaling pathway in the human chronic myelogenous leukemia cell line K562 and MEG-01 (Reuter, et al., 2009). NF- κ B and two Sp1 binding sites were involved in basal transcription of GGT in these cells (Reuter, et al., 2009). Ripple and colleagues treated LNCaP cells, an androgen responsive human prostate carcinoma cell line, with the synthetic androgen R1881 and found that GGT was induced at doses of R1881 that increased ROS in the cells (Ripple, Henry, Rago, & Wilding, 1997). Blocking the induction of ROS by ascorbic acid blocked the induction of GGT. In this study, treatment of LNCaP cells with H₂O₂ or menadione also induced GGT activity.

There is a large amount of publically available microarray data regarding expression of human GGT mRNA. However, studies of GGT mRNA levels in human tissues have been confounded by the expression of the a closely related gene GGT2 described above (see "OTHER GGT GENES") (Heisterkamp, et al., 2008). There is 97% nucleotide identity between GGT (a.k.a. GGT1) and GGT2, and 94% amino acid identity. The GGT2 gene is transcribed and GGT2 protein has been detected, but the protein has no enzymatic activity and is rapidly degraded within the cell (West, Wickham, et al., 2013). Some microarray platforms have probes that distinguish between GGT1 and GGT2, while others do not creating confusion as to which mRNA is being measured. As a result, microarray data for GGT mRNA expression in humans must be carefully evaluated based on the platform and probes used. In addition, some siRNAs that have been used to block translation of GGT2 have a high degree of homology to GGT1, such that functions of GGT1 have been ascribed to GGT2 (Moon, et al., 2012). Issues with GGT2 nomenclature, misidentified primers and siRNA have been summarized in detail (West, Wickham, et al., 2013). Due to these issues, only those studies that show an increase in both GGT mRNA and activity have been cited in this review with regard to induction of GGT in human cells.

GGT ACTIVITY IN SERUM

In humans, a low level of GGT activity can be detected in the serum of healthy individuals. GGT is a cell surface enzyme that is anchored in the membrane by a single transmembrane domain. People who have what is considered to be normal levels of GGT in serum (less than 60U/L) have a hydrophilic form of GGT in their serum. The source of this GGT is unclear. The level of GGT activity in the serum increases dramatically in patients with liver disease (Rosalki, 1975). Analysis of the GGT on non-denaturing gels shows that there are multiple forms of GGT in the serum of these patients including GGT bound to low density lipoproteins (Kojima et al., 1980; Paolicchi et al., 2006). When analyzed on non-denaturing

gels there is at least one unique form of GGT that is present in the serum of patients with hepatocellular carcinomas that is not present in patients with benign liver disease (Sawabu et al., 1983; Xu et al., 1992; Yao et al., 1998). The distinguishing characteristic is likely a glycan. We have reported tissue-specific glycosylation of GGT (West, et al., 2010). The unique tumor form of GGT may be useful as the basis of a serum assay to aid in the diagnosis of hepatocellular carcinoma (Kobata & Amano, 2005). In most cancer patients, except those with liver tumors, the level of GGT in serum does not increase even in patients with GGT-positive tumors unless the tumor (GGT-positive or GGT-negative) has metastasized to the liver (Whitfield, 2001). The low level of GGT in the serum of healthy individuals and most cancer patients may cleave some of the GSH in the serum. Nonetheless, the steady state level of GSH in human serum ranges from 5 to 20 μ M indicating that the GGT activity in the serum is too low to deplete the serum of GSH (Davis, et al., 2006).

OVERCOMING RESISTANCE TO PRO-OXIDANT THERAPY BY INHIBITING GGT

Reducing the intracellular GSH concentration sensitizes tumors to many diverse chemotherapy drugs (Butturini et al., 2013; Calvert, Yao, Hamilton, & O'Dwyer, 1998; Estrela, et al., 2006; Hamaguchi et al., 1993; Maciag et al., 2013; Ortega, et al., 2011; Ruoso & Hedley, 2004; Traverso et al., 2013) (Crook, Souhami, Whyman, & McLean, 1986). To date, two different approaches have been evaluated clinically to try to reduce the synthesis of intracellular GSH. Sulfasalazine, a drug that blocks the cystine transporter (but not the cysteine transporter) increased the sensitivity of human glioma cells to chemotherapy and inhibited growth of human prostate tumors in nude mice (Doxsee, et al., 2007; Pham, et al., 2010). Sulfasalazine selectively killed CD44v-positive tumor cells in mice bearing head and neck tumors (Yoshikawa, et al., 2013). Sulfasalazine is FDA-approved for use in the treatment of several conditions including inflammatory bowel disease. However, a phase I clinical trial using continuous daily dosing of sulfasalazine alone for the treatment of advanced gliomas proved neurotoxic and was terminated early (Robe et al., 2009). Buthionine sulfoximine (BSO) which inhibits the initial enzyme in GSH synthesis was evaluated in a Phase I trial with melphalan (Bailey et al., 1997). Continuous dosing of BSO for 72 hours in combination with melphalan resulted in severe myelosuppression. Neither of these treatments has advanced to clinical practice.

Inhibition of GGT is a novel approach to reducing intracellular GSH and sensitizing tumors to chemotherapy without the toxicities observed with sulfasalazine and BSO. Studies in mice have shown that administration of an inhibitor of GGT rapidly induces glutathionuria, decreases the cysteine levels in the tumor and reduces the intracellular concentration of GSH in tumors. One hour after administering an inhibitor of GGT to mice, the concentration of GSH in the urine increased 1800-fold (Griffith & Meister, 1979). Administration of a GGT inhibitor to a mouse bearing a cervical tumor resulted in a 50% drop in the cysteine concentration in the tumor within four hours (Ruoso & Hedley, 2004). Benlloch and colleagues showed that inhibiting GGT with acivicin in a model system of melanoma

metastasis to the liver in the presence of normal extracellular levels of cysteine, resulted in a 50% reduction in the GSH concentration in the tumors (Benlloch et al., 2005).

A significant difference between the effects of a GGT inhibitor and BSO is that when GGT is inhibited the GSH concentration in the serum increases (Griffith & Meister, 1979). The GSH concentration in the serum of wild-type mice is 28 μ M, while the serum of the GGT-knockout mice contains 175 μ M GSH (Lieberman, et al., 1996). It is not clear whether the increased concentration of GSH in the blood of GGT-knockout mice is due to increased synthesis and secretion of GSH by the liver or due to decreased catabolism of GSH as it circulates through the body (Anderson, Bridges, & Meister, 1980; Lauterburg, Adams, & Mitchell, 1984). However, cysteine depletion from serum that contains high levels of GSH does not appear to be toxic to blood cells as myelosuppression has not been observed in GGT-deficient mice (Lieberman, et al., 1996). In fact, inhibiting GGT has been shown to block the toxic side effects of several chemotherapy drugs. We discovered that inhibiting GGT protects against the dose-limiting nephrotoxicity of the widely used chemotherapy drug, cisplatin (M. H. Hanigan, Gallagher, et al., 1994; M. H. Hanigan, et al., 2001). We found that cisplatin is metabolized to a nephrotoxin via the GGT-dependent mercapturic acid pathway (Townsend, Deng, Zhang, Lopus, & Hanigan, 2003). Other investigators have shown that pulmonary fibrosis, a toxic side effect of bleomycin, is attenuated in GGT knockout mice and that surprisingly, it is the cysteine deficiency that is protective (Pardo et al., 2003).

GGT INHIBITORS

Tumors are under oxidative stress due to their increased metabolic rate (Pani, et al., 2010). They have higher intracellular GSH concentrations, utilize more cysteine than normal cells and therefore are more sensitive to cysteine depletion than normal cells. Administration of an inhibitor of GGT to a patient for several hours prior to therapy would reduce the supply of cysteine and cystine available to both GGT-positive and GGT-negative tumors. This would sensitize the tumors to cytotoxic chemotherapy, radiation and other pro-oxidant therapies. Systemic GGT inhibition would sensitize GGT-positive tumors not only by reducing cysteine concentrations in the blood, but also by blocking their unique ability to use extracellular GSH as an additional source of cysteine.

The most potent GGT inhibitors are glutamate analogs. They include acivicin, diazonorleucine and L-azaserine (Ahluwalia, Grem, Hao, & Cooney, 1990; Tate & Meister, 1978). However, they also inhibit glutamate metabolizing enzymes and, at doses needed to inhibit GGT, they are too toxic for clinical use (Ahluwalia, et al., 1990; Hidalgo et al., 1998). Two new classes of GGT inhibitors have been reported. One is gamma-phosphono glutamate analogs, which are irreversible inhibitors of human GGT (Han, Hiratake, Kamiyama, & Sakata, 2007). The lead compound, GGsTop, has been used to inhibit renal GGT in rats (Yamamoto, et al., 2011). These compounds are glutamate analogs and toxicity data have not been reported. We have identified a group of GGT inhibitors which are derivatives of our initial lead compound N-[5-(4-methoxybenzyl)-1,3,4-thiadiazol-2-yl]benzenesulfonamide, which we named OU749 (King, West, Cook, & Hanigan, 2009; Wickham et al., 2011; Wickham et al., 2013). These compounds are

uncompetitive inhibitors of human GGT with low toxicity and a large therapeutic window when evaluated in dividing human cells in culture (Wickham, et al., 2013). These inhibitors are under further development.

SUMMARY

Expression of GGT is essential in maintaining the cysteine levels in the body. Induction of GGT expression in response to redox stress provides the cell with access to additional cysteine, which becomes rate-limiting for intracellular GSH synthesis. Our understanding of the role of GGT in redox regulation and its induction in cells under redox stress will aid in the development of new anti-cancer therapies. GGT first came to the attention of cancer researchers as a biomarker for preneoplastic lesions in rodent models of hepatocarcinogenesis. Subsequent studies showed that the expression of GGT in the preneoplastic lesions provided a selective survival and proliferative advantage during treatment with promoting agents that deplete intracellular GSH. Clinical studies of GGT expression in human tumors revealed a correlation between GGT expression and poor patient survival. Expression of GGT on the entire surface of tumor cells provides the tumor with access to additional cysteine and cystine from GSH and GSSG in the interstitial fluid. This additional pool of cysteine allows the GGT-positive tumors to maintain higher levels of intracellular glutathione, enhancing their resistance to pro-oxidant therapy. Inhibition of GGT is a new approach to overcoming drug resistance in tumors. Inhibition of GGT several hours prior to the administration of chemotherapy would not only block the access of GGT-positive tumors to cysteine from extracellular GSH, but would also inhibit GGT in the kidney, resulting in glutathionuria and a rapid reduction in the levels of cysteine in the blood. As a result, GSH concentrations in both GGT-positive and GGT-negative tumors would decrease and the tumors would be sensitized to the therapy. Short term reduction of total body cysteine would not be detrimental to health, but would enhance the effectiveness of pro-oxidant chemotherapy. We have recently solved the crystal structure of human GGT which will accelerate the development of GGT inhibitors for clinical use.

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References

- Ahluwalia GS, Grem JL, Hao Z, Cooney DA. Metabolism and action of amino acid analog anti-cancer agents. *Pharmacol Ther.* 1990; 46(2):243–271. [PubMed: 2108451]
- Ahmad S, Okine L, Wood R, Aljian J, Vistica DT. gamma-Glutamyl transpeptidase (gamma-GT) and maintenance of thiol pools in tumor cells resistant to alkylating agents. *J Cell Physiol.* 1987; 131(2): 240–246. [PubMed: 2884224]
- Anders MW, Dekant W. Glutathione-dependent bioactivation of haloalkenes. *Annu Rev Pharmacol Toxicol.* 1998; 38:501–537. [PubMed: 9597164]
- Anderson ME, Bridges RJ, Meister A. Direct evidence for inter-organ transport of glutathione and that the non-filtration renal mechanism for glutathione utilization involves gamma-glutamyl transpeptidase. *Biochem Biophys Res Commun.* 1980; 96(2):848–853. [PubMed: 6107079]

- Auman JT, Church R, Lee SY, Watson MA, Fleshman JW, McLeod HL. Celecoxib pre-treatment in human colorectal adenocarcinoma patients is associated with gene expression alterations suggestive of diminished cellular proliferation. *Eur J Cancer*. 2008; 44(12):1754–1760. [PubMed: 18653328]
- Bachhawat AK, Thakur A, Kaur J, Zulkifli M. Glutathione transporters. *Biochim Biophys Acta*. 2013; 1830(5):3154–3164. [PubMed: 23206830]
- Bailey HH, Gipp JJ, Mulcahy RT. Increased expression of gamma-glutamyl transpeptidase in transfected tumor cells and its relationship to drug sensitivity. *Cancer Lett*. 1994; 87(2):163–170. [PubMed: 7812936]
- Bailey HH, Ripple G, Tutsch KD, Arzoomanian RZ, Alberti D, Feierabend C, et al. Phase I study of continuous-infusion L-S,R-buthionine sulfoximine with intravenous melphalan. *J Natl Cancer Inst*. 1997; 89(23):1789–1796. [PubMed: 9392620]
- Balendiran GK, Dabur R, Fraser D. The role of glutathione in cancer. *Cell Biochem Funct*. 2004; 22(6):343–352. [PubMed: 15386533]
- Bard S, Noel P, Chauvin F, Quash G. gamma-Glutamyltranspeptidase activity in human breast lesions: an unfavourable prognostic sign. *Br J Cancer*. 1986; 53(5):637–642. [PubMed: 2872909]
- Barrios R, Shi ZZ, Kala SV, Wiseman AL, Welty SE, Kala G, et al. Oxygen-induced pulmonary injury in gamma-glutamyl transpeptidase-deficient mice. *Lung*. 2001; 179(5):319–330. [PubMed: 11976899]
- Benlloch M, Ortega A, Ferrer P, Segarra R, Obrador E, Asensi M, et al. Acceleration of glutathione efflux and inhibition of gamma-glutamyltranspeptidase sensitize metastatic B16 melanoma cells to endothelium-induced cytotoxicity. *J Biol Chem*. 2005; 280(8):6950–6959. [PubMed: 15561710]
- Biddle A, Gammon L, Fazil B, Mackenzie IC. CD44 staining of cancer stem-like cells is influenced by down-regulation of CD44 variant isoforms and up-regulation of the standard CD44 isoform in the population of cells that have undergone epithelial-to-mesenchymal transition. *PLoS One*. 2013; 8(2):e57314. [PubMed: 23437366]
- Binkley F, Nakamura K. Metabolism of glutathione; hydrolysis by tissues of the rat. *J Biol Chem*. 1948; 173(1):411–421. [PubMed: 18902412]
- Blanco RA, Ziegler TR, Carlson BA, Cheng PY, Park Y, Cotsonis GA, et al. Diurnal variation in glutathione and cysteine redox states in human plasma. *Am J Clin Nutr*. 2007; 86(4):1016–1023. [PubMed: 17921379]
- Brannigan JA, Dodson G, Duggleby HJ, Moody PC, Smith JL, Tomchick DR, et al. A protein catalytic framework with an N-terminal nucleophile is capable of self-activation. *Nature*. 1995; 378(6555):416–419. [PubMed: 7477383]
- Brouillet A, Darbouy M, Okamoto T, Chobert MN, Lahuna O, Garlatti M, et al. Functional characterization of the rat gamma-glutamyl transpeptidase promoter that is expressed and regulated in the liver and hepatoma cells. *J Biol Chem*. 1994; 269(21):14878–14884. [PubMed: 7910821]
- Butturini E, de Prati AC, Chiavegato G, Rigo A, Cavalieri E, Darra E, et al. Mild oxidative stress induces S-glutathionylation of STAT3 and enhances chemosensitivity of tumoral cells to chemotherapeutic drugs. *Free Radic Biol Med*. 2013
- Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer*. 2011; 11(2):85–95. [PubMed: 21258394]
- Calvert P, Yao KS, Hamilton TC, O'Dwyer PJ. Clinical studies of reversal of drug resistance based on glutathione. *Chem Biol Interact*. 1998; 111–112:213–224.
- Cameron RG, Armstrong D, Gunsekara A, Varghese G, Speisky H. Utilization of circulating glutathione by nodular and cancerous intact rat liver. *Carcinogenesis*. 1991; 12(12):2369–2372. [PubMed: 1684139]
- Carter BZ, Shi ZZ, Barrios R, Lieberman MW. gamma-glutamyl leukotrienase, a gamma-glutamyl transpeptidase gene family member, is expressed primarily in spleen. *J Biol Chem*. 1998; 273(43):28277–28285. [PubMed: 9774450]
- Castonguay R, Halim D, Morin M, Furtos A, Lherbet C, Bonneil E, et al. Kinetic characterization and identification of the acylation and glycosylation sites of recombinant human gamma-glutamyltranspeptidase. *Biochemistry*. 2007; 46(43):12253–12262. [PubMed: 17924658]

- Chaiswing L, Zhong W, Oberley TD. Distinct Redox Profiles of Selected Human Prostate Carcinoma Cell Lines: Implications for Rational Design of Redox Therapy. *Cancers (Basel)*. 2011; 3(3):3557–3584. [PubMed: 22163073]
- Chaudhari P, Ye Z, Jang YY. Roles of Reactive Oxygen Species in the Fate of Stem Cells. *Antioxid Redox Signal*. 2012
- Chevez-Barrios P, Wiseman AL, Rojas E, Ou CN, Lieberman MW. Cataract development in gamma-glutamyl transpeptidase-deficient mice. *Exp Eye Res*. 2000; 71(6):575–582. [PubMed: 11095909]
- Chikhi N, Holic N, Guellaen G, Laperche Y. Gamma-glutamyl transpeptidase gene organization and expression: a comparative analysis in rat, mouse, pig and human species. *Comp Biochem Physiol B Biochem Mol Biol*. 1999; 122(4):367–380. [PubMed: 10392451]
- Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radic Biol Med*. 2010; 48(6):749–762. [PubMed: 20045723]
- Cole SP, Deeley RG. Transport of glutathione and glutathione conjugates by MRP1. *Trends Pharmacol Sci*. 2006; 27(8):438–446. [PubMed: 16820223]
- Cooper, AJL.; Hanigan, MH. Enzymes involved in processing glutathione conjugates. In: McQueen, CA., editor. *Comprehensive Toxicology*. Vol. 4. Oxford: Elsevier; 2010. p. 323–366. Chapter 17
- Corti A, Duarte TL, Giommarelli C, De Tata V, Paolicchi A, Jones GD, et al. Membrane gamma-glutamyl transferase activity promotes iron-dependent oxidative DNA damage in melanoma cells. *Mutat Res*. 2009; 669(1–2):112–121. [PubMed: 19505483]
- Courtay C, Heisterkamp N, Siest G, Groffen J. Expression of multiple gamma-glutamyltransferase genes in man. *Biochem J*. 1994; 297(Pt 3):503–508. [PubMed: 7906515]
- Crook TR, Souhami RL, Whyman GD, McLean AE. Glutathione depletion as a determinant of sensitivity of human leukemia cells to cyclophosphamide. *Cancer Res*. 1986; 46(10):5035–5038. [PubMed: 3463410]
- Curthoys NP, Hughey RP. Characterization and physiological function of rat renal gamma-glutamyltranspeptidase. *Enzyme*. 1979; 24(6):383–403. [PubMed: 42531]
- Daubeuf S, Accaoui MJ, Pettersen I, Huseby NE, Visvikis A, Galteau MM. Differential regulation of gamma-glutamyltransferase mRNAs in four human tumour cell lines. *Biochim Biophys Acta*. 2001; 1568(1):67–73. [PubMed: 11731087]
- Davis SR, Quinlivan EP, Stacpoole PW, Gregory JF 3rd. Plasma glutathione and cystathionine concentrations are elevated but cysteine flux is unchanged by dietary vitamin B-6 restriction in young men and women. *J Nutr*. 2006; 136(2):373–378. [PubMed: 16424114]
- DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, et al. Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature*. 2011; 475(7354):106–109. [PubMed: 21734707]
- Diederich M, Wellman M, Visvikis A, Puga A, Siest G. The 5' untranslated region of the human gamma-glutamyl transferase mRNA contains a tissue-specific active translational enhancer. *FEBS Lett*. 1993; 332(1–2):88–92. [PubMed: 8104826]
- Dominici S, Valentini M, Maellaro E, Del Bello B, Paolicchi A, Lorenzini E, et al. Redox modulation of cell surface protein thiols in U937 lymphoma cells: the role of gamma-glutamyl transpeptidase-dependent H₂O₂ production and S-thiolation. *Free Radic Biol Med*. 1999; 27(5–6):623–635. [PubMed: 10490284]
- Doxsee DW, Gout PW, Kurita T, Lo M, Buckley AR, Wang Y, et al. Sulfasalazine-induced cystine starvation: potential use for prostate cancer therapy. *Prostate*. 2007; 67(2):162–171. [PubMed: 17075799]
- Durham JR, Frierson HF Jr, Hanigan MH. Gamma-glutamyl transpeptidase immunoreactivity in benign and malignant breast tissue. *Breast Cancer Res Treat*. 1997; 45(1):55–62. [PubMed: 9285117]
- Estrela JM, Ortega A, Obrador E. Glutathione in cancer biology and therapy. *Crit Rev Clin Lab Sci*. 2006; 43(2):143–181. [PubMed: 16517421]
- Fagoonee S, Bearzi C, Di Cunto F, Clohessy JG, Rizzi R, Reschke M, et al. The RNA Binding Protein ESRP1 Fine-Tunes the Expression of Pluripotency-Related Factors in Mouse Embryonic Stem Cells. *PLoS One*. 2013; 8(8):e72300. [PubMed: 24015231]

- Figlewicz DA, Delattre O, Guellaen G, Krizus A, Thomas G, Zucman J, et al. Mapping of human gamma-glutamyl transpeptidase genes on chromosome 22 and other human autosomes. *Genomics*. 1993; 17(2):299–305. [PubMed: 8104871]
- Forman HJ, Skelton DC. Protection of alveolar macrophages from hyperoxia by gamma-glutamyl transpeptidase. *Am J Physiol*. 1990; 259(2 Pt 1):L102–107. [PubMed: 1974390]
- Gallagher BC, Rudolph DB, Hinton BT, Hanigan MH. Differential induction of gamma-glutamyl transpeptidase in primary cultures of rat and mouse hepatocytes parallels induction during hepatocarcinogenesis. *Carcinogenesis*. 1998; 19(7):1251–1255. [PubMed: 9683185]
- Galperin MY, Koonin EV. Divergence and convergence in enzyme evolution. *J Biol Chem*. 2012; 287(1):21–28. [PubMed: 22069324]
- Glass GA, Stark AA. Promotion of glutathione-gamma-glutamyl transpeptidase-dependent lipid peroxidation by copper and ceruloplasmin: the requirement for iron and the effects of antioxidants and antioxidant enzymes. *Environ Mol Mutagen*. 1997; 29(1):73–80. [PubMed: 9020310]
- Go YM, Jones DP. Thiol/disulfide redox states in signaling and sensing. *Crit Rev Biochem Mol Biol*. 2013; 48(2):173–181. [PubMed: 23356510]
- Godwin AK, Meister A, O'Dwyer PJ, Huang CS, Hamilton TC, Anderson ME. High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis. *Proc Natl Acad Sci U S A*. 1992; 89(7):3070–3074. [PubMed: 1348364]
- Goldsworthy TL, Hanigan MH, Pitot HC. Models of hepatocarcinogenesis in the rat--contrasts and comparisons. *Crit Rev Toxicol*. 1986; 17(1):61–89. [PubMed: 3527567]
- Griffith OW, Meister A. Translocation of intracellular glutathione to membrane-bound gamma-glutamyl transpeptidase as a discrete step in the gamma-glutamyl cycle: glutathionuria after inhibition of transpeptidase. *Proc Natl Acad Sci U S A*. 1979; 76(1):268–272. [PubMed: 34150]
- Grimm C, Hofstetter G, Aust S, Mutz-Dehbalae I, Bruch M, Heinze G, et al. Association of gamma-glutamyltransferase with severity of disease at diagnosis and prognosis of ovarian cancer. *Br J Cancer*. 2013; 109(3):610–614. [PubMed: 23921280]
- Hamaguchi K, Godwin AK, Yakushiji M, O'Dwyer PJ, Ozols RF, Hamilton TC. Cross-resistance to diverse drugs is associated with primary cisplatin resistance in ovarian cancer cell lines. *Cancer Res*. 1993; 53(21):5225–5232. [PubMed: 8106143]
- Han L, Hiratake J, Kamiyama A, Sakata K. Design, synthesis, and evaluation of gamma-phosphono diester analogues of glutamate as highly potent inhibitors and active site probes of gamma-glutamyl transpeptidase. *Biochemistry*. 2007; 46(5):1432–1447. [PubMed: 17260973]
- Hanes CS, Hird FJ, Isherwood FA. Enzymic transpeptidation reactions involving gamma-glutamyl peptides and alpha-amino-acyl peptides. *Biochem J*. 1952; 51(1):25–35. [PubMed: 14944528]
- Hanigan HM, Laishes BA. Toxicity of aflatoxin B1 in rat and mouse hepatocytes in vivo and in vitro. *Toxicology*. 1984; 30(3):185–193. [PubMed: 6424269]
- Hanigan MH. Expression of gamma-glutamyl transpeptidase provides tumor cells with a selective growth advantage at physiologic concentrations of cyst(e)ine. *Carcinogenesis*. 1995; 16(2):181–185. [PubMed: 7859346]
- Hanigan MH, Brown JE, Ricketts WA. Gamma-glutamyl transpeptidase, a glutathionase, is present in some cell culture grade bovine sera. *In Vitro Cell Dev Biol Anim*. 1993; 29A(11):831–833. [PubMed: 7909487]
- Hanigan MH, Frierson HF Jr. Immunohistochemical detection of gamma-glutamyl transpeptidase in normal human tissue. *J Histochem Cytochem*. 1996; 44(10):1101–1108. [PubMed: 8813074]
- Hanigan MH, Frierson HF Jr, Brown JE, Lovell MA, Taylor PT. Human ovarian tumors express gamma-glutamyl transpeptidase. *Cancer Res*. 1994; 54(1):286–290. [PubMed: 7903206]
- Hanigan MH, Frierson HF Jr, Swanson PE, De Young BR. Altered expression of gamma-glutamyl transpeptidase in human tumors. *Hum Pathol*. 1999; 30(3):300–305. [PubMed: 10088549]
- Hanigan MH, Frierson HF Jr, Taylor PT Jr. Expression of gamma-glutamyl transpeptidase in stage III and IV ovarian surface epithelial carcinomas does not alter response to primary cisplatin-based chemotherapy. *Am J Obstet Gynecol*. 1998; 179(2):363–367. [PubMed: 9731839]
- Hanigan MH, Gallagher BC, Taylor PT Jr, Large MK. Inhibition of gamma-glutamyl transpeptidase activity by acivicin in vivo protects the kidney from cisplatin-induced toxicity. *Cancer Res*. 1994; 54(22):5925–5929. [PubMed: 7954424]

- Hanigan MH, Gallagher BC, Townsend DM, Gabarra V. Gamma-glutamyl transpeptidase accelerates tumor growth and increases the resistance of tumors to cisplatin in vivo. *Carcinogenesis*. 1999; 20(4):553–559. [PubMed: 10223181]
- Hanigan MH, Lykissa ED, Townsend DM, Ou CN, Barrios R, Lieberman MW. Gamma-glutamyl transpeptidase-deficient mice are resistant to the nephrotoxic effects of cisplatin. *Am J Pathol*. 2001; 159(5):1889–1894. [PubMed: 11696449]
- Hanigan MH, Pitot HC. Activities of benzphetamine N-demethylase and aryl hydrocarbon hydroxylase in cells isolated from gamma-glutamyl transpeptidase-positive foci and surrounding liver. *J Natl Cancer Inst*. 1985a; 75(6):1107–1112. [PubMed: 2866268]
- Hanigan MH, Pitot HC. Gamma-glutamyl transpeptidase--its role in hepatocarcinogenesis. *Carcinogenesis*. 1985b; 6(2):165–172. [PubMed: 2857599]
- Hanigan MH, Ricketts WA. Extracellular glutathione is a source of cysteine for cells that express gamma-glutamyl transpeptidase. *Biochemistry*. 1993; 32(24):6302–6306. [PubMed: 8099811]
- Hanigan MH, Winkler ML, Drinkwater NR. Induction of three histochemically distinct populations of hepatic foci in C57BL/6J mice. *Carcinogenesis*. 1993; 14(5):1035–1040. [PubMed: 8099313]
- Harding CO, Williams P, Wagner E, Chang DS, Wild K, Colwell RE, et al. Mice with genetic gamma-glutamyl transpeptidase deficiency exhibit glutathionuria, severe growth failure, reduced life spans, and infertility. *J Biol Chem*. 1997; 272(19):12560–12567. [PubMed: 9139708]
- Hayes JD, Judah DJ, Neal GE, Nguyen T. Molecular cloning and heterologous expression of a cDNA encoding a mouse glutathione S-transferase Yc subunit possessing high catalytic activity for aflatoxin B1-8,9-epoxide. *Biochem J*. 1992; 285(Pt 1):173–180. [PubMed: 1637297]
- Heisterkamp N, Groffen J, Warburton D, Sneddon TP. The human gamma-glutamyltransferase gene family. *Hum Genet*. 2008; 123(4):321–332. [PubMed: 18357469]
- Heisterkamp N, Rajpert-De Meyts E, Uribe L, Forman HJ, Groffen J. Identification of a human gamma-glutamyl cleaving enzyme related to, but distinct from, gamma-glutamyl transpeptidase. *Proc Natl Acad Sci U S A*. 1991; 88(14):6303–6307. [PubMed: 1676842]
- Hidalgo M, Rodriguez G, Kuhn JG, Brown T, Weiss G, MacGovren JP, et al. A Phase I and pharmacological study of the glutamine antagonist acivicin with the amino acid solution aminosyn in patients with advanced solid malignancies. *Clin Cancer Res*. 1998; 4(11):2763–2770. [PubMed: 9829740]
- Hochwald SN, Harrison LE, Rose DM, Anderson M, Burt ME. gamma-Glutamyl transpeptidase mediation of tumor glutathione utilization in vivo. *J Natl Cancer Inst*. 1996; 88(3–4):193–197. [PubMed: 8632493]
- Hochwald SN, Rose DM, Brennan MF, Burt ME. Elevation of glutathione and related enzyme activities in high-grade and metastatic extremity soft tissue sarcoma. *Ann Surg Oncol*. 1997; 4(4):303–309. [PubMed: 9181229]
- Huseby NE, Asare N, Wetting S, Mikkelsen IM, Mortensen B, Wellman M. Role of gamma-glutamyltransferase in the homeostasis of glutathione during oxidative and nitrosative stress. *Biofactors*. 2003; 17(1–4):151–160. [PubMed: 12897437]
- Ikeda Y, Fujii J, Taniguchi N, Meister A. Expression of an active glycosylated human gamma-glutamyl transpeptidase mutant that lacks a membrane anchor domain. *Proc Natl Acad Sci U S A*. 1995; 92(1):126–130. [PubMed: 7816801]
- Ishimoto T, Nagano O, Yae T, Tamada M, Motohara T, Oshima H, et al. CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc(-) and thereby promotes tumor growth. *Cancer Cell*. 2011; 19(3):387–400. [PubMed: 21397861]
- Jean JC, Liu Y, Brown LA, Marc RE, Klings E, Joyce-Brady M. Gamma-glutamyl transferase deficiency results in lung oxidant stress in normoxia. *Am J Physiol Lung Cell Mol Physiol*. 2002; 283(4):L766–776. [PubMed: 12225953]
- Jones DP. Redox sensing: orthogonal control in cell cycle and apoptosis signalling. *J Intern Med*. 2010; 268(5):432–448. [PubMed: 20964735]
- Joyce-Brady M, Jean JC, Hughey RP. gamma -glutamyltransferase and its isoform mediate an endoplasmic reticulum stress response. *J Biol Chem*. 2001; 276(12):9468–9477. [PubMed: 11116135]

- Kang JS, Wanibuchi H, Morimura K, Wongpoomchai R, Chusiri Y, Gonzalez FJ, et al. Role of CYP2E1 in thioacetamide-induced mouse hepatotoxicity. *Toxicol Appl Pharmacol.* 2008; 228(3): 295–300. [PubMed: 18374380]
- Kansanen E, Kuosmanen SM, Leinonen H, Levonen AL. The Keap1-Nrf2 pathway: Mechanisms of activation and dysregulation in cancer. *Redox Biol.* 2013; 1(1):45–49. [PubMed: 24024136]
- Karp DR, Shimooku K, Lipsky PE. Expression of gamma-glutamyl transpeptidase protects ramos B cells from oxidation-induced cell death. *J Biol Chem.* 2001; 276(6):3798–3804. [PubMed: 11080500]
- Keillor JW, Castonguay R, Lherbet C. Gamma-glutamyl transpeptidase substrate specificity and catalytic mechanism. *Methods Enzymol.* 2005; 401:449–467. [PubMed: 16399402]
- King JB, West MB, Cook PF, Hanigan MH. A novel, species-specific class of uncompetitive inhibitors of gamma-glutamyl transpeptidase. *J Biol Chem.* 2009; 284(14):9059–9065. [PubMed: 19203993]
- Kinoshita H, Okabe H, Beppu T, Chikamoto A, Hayashi H, Imai K, et al. Cystine/glutamic acid transporter is a novel marker for predicting poor survival in patients with hepatocellular carcinoma. *Oncol Rep.* 2013; 29(2):685–689. [PubMed: 23229496]
- Knickelbein RG, Ingbar DH, Seres T, Snow K, Johnston RB Jr, Fayemi O, et al. Hyperoxia enhances expression of gamma-glutamyl transpeptidase and increases protein S-glutathiolation in rat lung. *Am J Physiol.* 1996; 270(1 Pt 1):L115–122. [PubMed: 8772534]
- Kobata A, Amano J. Altered glycosylation of proteins produced by malignant cells, and application for the diagnosis and immunotherapy of tumours. *Immunol Cell Biol.* 2005; 83(4):429–439. [PubMed: 16033539]
- Kojima J, Kanatani M, Nakamura N, Kashiwagi T, Tohjoh F, Akiyama M. Electrophoretic fractionation of serum gamma-glutamyl transpeptidase in human hepatic cancer. *Clin Chim Acta.* 1980; 106(2):165–172. [PubMed: 6157503]
- Kwiecien I, Rokita H, Lorenc-Koci E, Sokolowska M, Wlodek L. The effect of modulation of gamma-glutamyl transpeptidase and nitric oxide synthase activity on GSH homeostasis in HepG2 cells. *Fundam Clin Pharmacol.* 2007; 21(1):95–103. [PubMed: 17227450]
- Lauterburg BH, Adams JD, Mitchell JR. Hepatic glutathione homeostasis in the rat: efflux accounts for glutathione turnover. *Hepatology.* 1984; 4(4):586–590. [PubMed: 6745847]
- Lewerenz J, Hewett SJ, Huang Y, Lambros M, Gout PW, Kalivas PW, et al. The cystine/glutamate antiporter system x(c)(-) in health and disease: from molecular mechanisms to novel therapeutic opportunities. *Antioxid Redox Signal.* 2013; 18(5):522–555. [PubMed: 22667998]
- Lewis AD, Hayes JD, Wolf CR. Glutathione and glutathione-dependent enzymes in ovarian adenocarcinoma cell lines derived from a patient before and after the onset of drug resistance: intrinsic differences and cell cycle effects. *Carcinogenesis.* 1988; 9(7):1283–1287. [PubMed: 2898306]
- Lieberman MW, Barrios R, Carter BZ, Habib GM, Lebovitz RM, Rajagopalan S, et al. gamma-Glutamyl transpeptidase. What does the organization and expression of a multipromoter gene tell us about its functions? *Am J Pathol.* 1995; 147(5):1175–1185. [PubMed: 7485380]
- Lieberman MW, Wiseman AL, Shi ZZ, Carter BZ, Barrios R, Ou CN, et al. Growth retardation and cysteine deficiency in gamma-glutamyl transpeptidase-deficient mice. *Proc Natl Acad Sci U S A.* 1996; 93(15):7923–7926. [PubMed: 8755578]
- Maciag AE, Holland RJ, Robert Cheng YS, Rodriguez LG, Saavedra JE, Anderson LM, et al. Nitric oxide-releasing prodrug triggers cancer cell death through deregulation of cellular redox balance. *Redox Biol.* 2013; 1(1):115–124. [PubMed: 24024144]
- Mandel HG, Manson MM, Judah DJ, Simpson JL, Green JA, Forrester LM, et al. Metabolic basis for the protective effect of the antioxidant ethoxyquin on aflatoxin B1 hepatocarcinogenesis in the rat. *Cancer Res.* 1987; 47(19):5218–5223. [PubMed: 2887284]
- Meister A. On the enzymology of amino acid transport. *Science.* 1973; 180(4081):33–39. [PubMed: 4144403]
- Meister A. Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. *Pharmacol Ther.* 1991; 51(2):155–194. [PubMed: 1784629]
- Meredith MJ. Cystathionase activity and glutathione metabolism in redifferentiating rat hepatocyte primary cultures. *Cell Biol Toxicol.* 1987; 3(4):361–377. [PubMed: 3507263]

- Mikkelsen IM, Mortensen B, Laperche Y, Huseby NE. The expression of gamma-glutamyltransferase in rat colon carcinoma cells is distinctly regulated during differentiation and oxidative stress. *Mol Cell Biochem.* 2002; 232(1–2):87–95. [PubMed: 12030384]
- Mistry P, Harrap KR. Historical aspects of glutathione and cancer chemotherapy. *Pharmacol Ther.* 1991; 49(1–2):125–132. [PubMed: 1852785]
- Moon DO, Kim BY, Jang JH, Kim MO, Jayasooriya RG, Kang CH, et al. K-RAS transformation in prostate epithelial cell overcomes H₂O₂-induced apoptosis via upregulation of gamma-glutamyltransferase-2. *Toxicol In Vitro.* 2012; 26(3):429–434. [PubMed: 22269385]
- Moriarty-Craige SE, Jones DP. Extracellular thiols and thiol/disulfide redox in metabolism. *Annu Rev Nutr.* 2004; 24:481–509. [PubMed: 15189129]
- Morris C, Courtay C, Geurts van Kessel A, ten Hoeve J, Heisterkamp N, Groffen J. Localization of a gamma-glutamyl-transferase-related gene family on chromosome 22. *Hum Genet.* 1993; 91(1):31–36. [PubMed: 8095916]
- Morrow AL, Williams K, Sand A, Boanca G, Barycki JJ. Characterization of *Helicobacter pylori* gamma-glutamyltranspeptidase reveals the molecular basis for substrate specificity and a critical role for the tyrosine 433-containing loop in catalysis. *Biochemistry.* 2007; 46(46):13407–13414. [PubMed: 17960917]
- Nagano O, Okazaki S, Saya H. Redox regulation in stem-like cancer cells by CD44 variant isoforms. *Oncogene.* 2013
- Nakagawa Y, Suzuki T, Nakajima K, Ishii H, Ogata A. Biotransformation and cytotoxic effects of hydroxychavicol, an intermediate of safrole metabolism, in isolated rat hepatocytes. *Chem Biol Interact.* 2009; 180(1):89–97. [PubMed: 19428348]
- Oguchi H, Kikkawa F, Kojima M, Maeda O, Mizuno K, Suganuma N, et al. Glutathione related enzymes in cis-diamminedichloroplatinum (II)-sensitive and-resistant human ovarian carcinoma cells. *Anticancer Res.* 1994; 14(1A):193–200. [PubMed: 7909418]
- Oinonen C, Rouvinen J. Structural comparison of Ntn-hydrolases. *Protein Sci.* 2000; 9(12):2329–2337. [PubMed: 11206054]
- Okada T, Suzuki H, Wada K, Kumagai H, Fukuyama K. Crystal structures of gamma-glutamyltranspeptidase from *Escherichia coli*, a key enzyme in glutathione metabolism, and its reaction intermediate. *Proc Natl Acad Sci U S A.* 2006; 103(17):6471–6476. [PubMed: 16618936]
- Orlowski M, Meister A. Gamma-Glutamyl-P-Nitroanilide: A New Convenient Substrate for Determination and Study of L- and D-Gamma-Glutamyltranspeptidase Activities. *Biochim Biophys Acta.* 1963; 73:679–681. [PubMed: 14074151]
- Orlowski M, Meister A. The gamma-glutamyl cycle: a possible transport system for amino acids. *Proc Natl Acad Sci U S A.* 1970; 67(3):1248–1255. [PubMed: 5274454]
- Ortega AL, Salvador M, Estrela JM. Glutathione in Cancer Cell Death. *Cancers (Basel).* 2011; 3:1285–1310. [PubMed: 24212662]
- Pandur S, Pankiv S, Johannessen M, Moens U, Huseby NE. Gamma-glutamyltransferase is upregulated after oxidative stress through the Ras signal transduction pathway in rat colon carcinoma cells. *Free Radic Res.* 2007; 41(12):1376–1384. [PubMed: 18075840]
- Pani G, Galeotti T, Chiarugi P. Metastasis: cancer cell's escape from oxidative stress. *Cancer Metastasis Rev.* 2010; 29(2):351–378. [PubMed: 20386957]
- Pankiv S, Moller S, Bjorkoy G, Moens U, Huseby NE. Radiation-induced upregulation of gamma-glutamyltransferase in colon carcinoma cells is mediated through the Ras signal transduction pathway. *Biochim Biophys Acta.* 2006; 1760(2):151–157. [PubMed: 16386375]
- Paolicchi A, Emdin M, Passino C, Lorenzini E, Titta F, Marchi S, et al. Beta-lipoprotein- and LDL-associated serum gamma-glutamyltransferase in patients with coronary atherosclerosis. *Atherosclerosis.* 2006; 186(1):80–85. [PubMed: 16112119]
- Pardo A, Ruiz V, Arreola JL, Ramirez R, Cisneros-Lira J, Gaxiola M, et al. Bleomycin-induced pulmonary fibrosis is attenuated in gamma-glutamyl transpeptidase-deficient mice. *Am J Respir Crit Care Med.* 2003; 167(6):925–932. [PubMed: 12468440]
- PetitClerc C, Shiele F, Bagrel D, Mahassen A, Siest G. Kinetic properties of gamma-glutamyltransferase from human liver. *Clin Chem.* 1980; 26(12):1688–1693. [PubMed: 6107181]

- Pham AN, Blower PE, Alvarado O, Ravula R, Gout PW, Huang Y. Pharmacogenomic approach reveals a role for the x(c)- cystine/glutamate antiporter in growth and celastrol resistance of glioma cell lines. *J Pharmacol Exp Ther.* 2010; 332(3):949–958. [PubMed: 20007406]
- Rajpert-De Meyts E, Heisterkamp N, Groffen J. Cloning and nucleotide sequence of human gamma-glutamyl transpeptidase. *Proc Natl Acad Sci U S A.* 1988; 85(23):8840–8844. [PubMed: 2904146]
- Rajpert-De Meyts E, Shi M, Chang M, Robison TW, Groffen J, Heisterkamp N, et al. Transfection with gamma-glutamyl transpeptidase enhances recovery from glutathione depletion using extracellular glutathione. *Toxicol Appl Pharmacol.* 1992; 114(1):56–62. [PubMed: 1350117]
- Rao MS, Lalwani ND, Scarpelli DG, Reddy JK. The absence of gamma-glutamyl transpeptidase activity in putative preneoplastic lesions and in hepatocellular carcinomas induced in rats by the hypolipidemic peroxisome proliferator Wy-14,643. *Carcinogenesis.* 1982; 3(10):1231–1233. [PubMed: 6129072]
- Ravuri C, Svineng G, Pankiv S, Huseby NE. Endogenous production of reactive oxygen species by the NADPH oxidase complexes is a determinant of gamma-glutamyltransferase expression. *Free Radic Res.* 2011; 45(5):600–610. [PubMed: 21381898]
- Reuter S, Schnekenburger M, Cristofanon S, Buck I, Teiten MH, Daubeuf S, et al. Tumor necrosis factor alpha induces gamma-glutamyltransferase expression via nuclear factor-kappaB in cooperation with Sp1. *Biochem Pharmacol.* 2009; 77(3):397–411. [PubMed: 18996094]
- Ripple MO, Henry WF, Rago RP, Wilding G. Prooxidant-antioxidant shift induced by androgen treatment of human prostate carcinoma cells. *J Natl Cancer Inst.* 1997; 89(1):40–48. [PubMed: 8978405]
- Robe PA, Martin DH, Nguyen-Khac MT, Artesi M, Deprez M, Albert A, et al. Early termination of ISRCTN45828668, a phase 1/2 prospective, randomized study of sulfasalazine for the treatment of progressing malignant gliomas in adults. *BMC Cancer.* 2009; 9:372. [PubMed: 19840379]
- Rojas E, Valverde M, Kala SV, Kala G, Lieberman MW. Accumulation of DNA damage in the organs of mice deficient in gamma-glutamyltranspeptidase. *Mutat Res.* 2000; 447(2):305–316. [PubMed: 10751614]
- Roomi MW, Gaal K, Yuan QX, French BA, Fu P, Bardag-Gorce F, et al. Preneoplastic liver cell foci expansion induced by thioacetamide toxicity in drug-primed mice. *Exp Mol Pathol.* 2006; 81(1): 8–14. [PubMed: 16729998]
- Rosalki SB. Gamma-glutamyl transpeptidase. *Adv Clin Chem.* 1975; 17:53–107. [PubMed: 236637]
- Ruosio P, Hedley DW. Inhibition of gamma-glutamyl transpeptidase activity decreases intracellular cysteine levels in cervical carcinoma. *Cancer Chemother Pharmacol.* 2004; 54(1):49–56. [PubMed: 15034755]
- Sawabu N, Nakagen M, Ozaki K, Wakabayashi T, Toya D, Hattori N, et al. Clinical evaluation of specific gamma-GTP isoenzyme in patients with hepatocellular carcinoma. *Cancer.* 1983; 51(2): 327–331. [PubMed: 6185200]
- Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med.* 2001; 30(11):1191–1212. [PubMed: 11368918]
- Schulman JD, Goodman SI, Mace JW, Patrick AD, Tietze F, Butler EJ. Glutathionuria: inborn error of metabolism due to tissue deficiency of gamma-glutamyl transpeptidase. *Biochem Biophys Res Commun.* 1975; 65(1):68–74. [PubMed: 238530]
- Scopelliti AJ, Ryan RM, Vandenberg RJ. Molecular determinants for functional differences between alanine-serine-cysteine transporter 1 and other glutamate transporter family members. *J Biol Chem.* 2013; 288(12):8250–8257. [PubMed: 23393130]
- Shi M, Gozal E, Choy HA, Forman HJ. Extracellular glutathione and gamma-glutamyl transpeptidase prevent H₂O₂-induced injury by 2,3-dimethoxy-1,4-naphthoquinone. *Free Radic Biol Med.* 1993; 15(1):57–67. [PubMed: 8103030]
- Shi ZZ, Han B, Habib GM, Matzuk MM, Lieberman MW. Disruption of gamma-glutamyl leukotrienase results in disruption of leukotriene D(4) synthesis in vivo and attenuation of the acute inflammatory response. *Mol Cell Biol.* 2001; 21(16):5389–5395. [PubMed: 11463821]

- Sies H, Bartoli GM, Burk RF, Waydhas C. Glutathione efflux from perfused rat liver after phenobarbital treatment, during drug oxidations, and in selenium deficiency. *Eur J Biochem.* 1978; 89(1):113–118. [PubMed: 699901]
- Slaga TJ, Budunova IV, Gimenez-Conti IB, Aldaz CM. The mouse skin carcinogenesis model. *J Investig Dermatol Symp Proc.* 1996; 1(2):151–156.
- Stark AA, Russell JJ, Langenbach R, Pagano DA, Zeiger E, Huberman E. Localization of oxidative damage by a glutathione-gamma-glutamyl transpeptidase system in preneoplastic lesions in sections of livers from carcinogen-treated rats. *Carcinogenesis.* 1994; 15(2):343–348. [PubMed: 7906207]
- Stark AA, Zeiger E, Pagano DA. Glutathione metabolism by gamma-glutamyltranspeptidase leads to lipid peroxidation: characterization of the system and relevance to hepatocarcinogenesis. *Carcinogenesis.* 1993; 14(2):183–189. [PubMed: 8094645]
- Stenius U, Rubin K, Gullberg D, Hogberg J. gamma-Glutamyltranspeptidase-positive rat hepatocytes are protected from GSH depletion, oxidative stress and reversible alterations of collagen receptors. *Carcinogenesis.* 1990; 11(1):69–73. [PubMed: 1967230]
- Takahashi Y, Oakes SM, Williams MC, Takahashi S, Miura T, Joyce-Brady M. Nitrogen dioxide exposure activates gamma-glutamyl transferase gene expression in rat lung. *Toxicol Appl Pharmacol.* 1997; 143(2):388–396. [PubMed: 9144455]
- Takahashi Y, Takahashi S, Yoshimi T, Miura T, Mochitate K, Kobayashi T. Increases in the mRNA levels of gamma-glutamyltransferase and heme oxygenase-1 in the rat lung after ozone exposure. *Biochem Pharmacol.* 1997; 53(7):1061–1064. [PubMed: 9174121]
- Takeuchi S, Wada K, Toyooka T, Shinomiya N, Shimazaki H, Nakanishi K, et al. Increased xCT expression correlates with tumor invasion and outcome in patients with glioblastomas. *Neurosurgery.* 2013; 72(1):33–41. discussion 41. [PubMed: 23096413]
- Tate SS, Meister A. Serine-borate complex as a transition-state inhibitor of gamma-glutamyl transpeptidase. *Proc Natl Acad Sci U S A.* 1978; 75(10):4806–4809. [PubMed: 33382]
- Tateishi N, Higashi T, Nomura T, Naruse A, Nakashima K. Higher transpeptidation activity and broad acceptor specificity of gamma-glutamyltransferases of tumors. *Gann.* 1976; 67(2):215–222. [PubMed: 9327]
- Tew KD, Townsend DM. Glutathione-S-transferases as determinants of cell survival and death. *Antioxid Redox Signal.* 2012; 17(12):1728–1737. [PubMed: 22540427]
- Townsend DM, Deng M, Zhang L, Lapus MG, Hanigan MH. Metabolism of Cisplatin to a nephrotoxin in proximal tubule cells. *J Am Soc Nephrol.* 2003; 14(1):1–10. [PubMed: 12506132]
- Townsend DM, Hanigan MH. Inhibition of gamma-glutamyl transpeptidase or cysteine S-conjugate beta-lyase activity blocks the nephrotoxicity of cisplatin in mice. *J Pharmacol Exp Ther.* 2002; 300(1):142–148. [PubMed: 11752109]
- Traverso N, Ricciarelli R, Nitti M, Marengo B, Furfaro AL, Pronzato MA, et al. Role of glutathione in cancer progression and chemoresistance. *Oxid Med Cell Longev.* 2013; 2013:972913. [PubMed: 23766865]
- Vassallo JD, Hicks SM, Born SL, Daston GP. Roles for epoxidation and detoxification of coumarin in determining species differences in clara cell toxicity. *Toxicol Sci.* 2004; 82(1):26–33. [PubMed: 15282406]
- Vassallo JD, Kaetzel RS, Born SL, Lewis CL, Lehman-McKeeman LD, Reed DJ. Gamma-glutamyl transpeptidase null mice fail to develop tolerance to coumarin-induced Clara cell toxicity. *Food Chem Toxicol.* 2010; 48(6):1612–1618. [PubMed: 20347914]
- Vene R, Castellani P, Delfino L, Lucibello M, Ciriolo MR, Rubartelli A. The cystine/cysteine cycle and GSH are independent and crucial antioxidant systems in malignant melanoma cells and represent druggable targets. *Antioxid Redox Signal.* 2011; 15(9):2439–2453. [PubMed: 21529243]
- Vina J, Perez C, Furukawa T, Palacin M, Vina JR. Effect of oral glutathione on hepatic glutathione levels in rats and mice. *Br J Nutr.* 1989; 62(3):683–691. [PubMed: 2605158]
- Visvikis A, Pawlak A, Accaoui MJ, Ichino K, Leh H, Guellaen G, et al. Structure of the 5' sequences of the human gamma-glutamyltransferase gene. *Eur J Biochem.* 2001; 268(2):317–325. [PubMed: 11168366]

- Warren DL, Brown DL Jr, Buckpitt AR. Evidence for cytochrome P-450 mediated metabolism in the bronchiolar damage by naphthalene. *Chem Biol Interact.* 1982; 40(3):287–303. [PubMed: 7083396]
- Warzecha CC, Shen S, Xing Y, Carstens RP. The epithelial splicing factors ESRP1 and ESRP2 positively and negatively regulate diverse types of alternative splicing events. *RNA Biol.* 2009; 6(5):546–562. [PubMed: 19829082]
- West MB, Chen Y, Wickham S, Heroux A, Cahill K, Hanigan MH, et al. Novel insights into eukaryotic gamma-glutamyl transpeptidase 1 from the crystal structure of the glutamate-bound human enzyme. *J Biol Chem.* 2013; 288(44):31902–31913. [PubMed: 24047895]
- West MB, Segu ZM, Feasley CL, Kang P, Klouckova I, Li C, et al. Analysis of site-specific glycosylation of renal and hepatic gamma-glutamyl transpeptidase from normal human tissue. *J Biol Chem.* 2010; 285(38):29511–29524. [PubMed: 20622017]
- West MB, Wickham S, Parks EE, Sherry DM, Hanigan MH. Human GGT2 Does Not Autocleave into a Functional Enzyme: A Cautionary Tale for Interpretation of Microarray Data on Redox Signaling. *Antioxid Redox Signal.* 2013; 19(16):1877–1888. [PubMed: 23682772]
- West MB, Wickham S, Quinalty LM, Pavlovicz RE, Li C, Hanigan MH. Autocatalytic Cleavage of Human {gamma}-Glutamyl Transpeptidase Is Highly Dependent on N-Glycosylation at Asparagine 95. *J Biol Chem.* 2011; 286(33):28876–28888. [PubMed: 21712391]
- Whitfield JB. Gamma glutamyl transferase. *Crit Rev Clin Lab Sci.* 2001; 38(4):263–355. [PubMed: 11563810]
- Wickham S, Regan N, West MB, Kumar VP, Thai J, Li PK, et al. Divergent effects of compounds on the hydrolysis and transpeptidation reactions of gamma-glutamyl transpeptidase. *J Enzyme Inhib Med Chem.* 2011
- Wickham S, Regan N, West MB, Thai J, Cook PF, Terzyan SS, et al. Inhibition of human gamma-glutamyl transpeptidase: development of more potent, physiologically relevant, uncompetitive inhibitors. *Biochem J.* 2013; 450(3):547–557. [PubMed: 23301618]
- Wickham S, West MB, Cook PF, Hanigan MH. Gamma-glutamyl compounds: Substrate specificity of gamma-glutamyl transpeptidase enzymes. *Anal Biochem.* 2011; 414:208–214. [PubMed: 21447318]
- Williams GM, Ohmori T, Katayama S, Rice JM. Alteration by phenobarbital of membrane-associated enzymes including gamma glutamyl transpeptidase in mouse liver neoplasms. *Carcinogenesis.* 1980; 1(10):813–818. [PubMed: 11219852]
- Woodlock TJ, Brown R, Mani M, Pompeo L, Hoffman H, Segel GB, et al. Decreased L system amino acid transport and decreased gamma-glutamyl transpeptidase are independent processes in human chronic lymphocytic leukemia B-lymphocytes. *J Cell Physiol.* 1990; 145(2):217–221. [PubMed: 1978835]
- Wright EC, Stern J, Ersser R, Patrick AD. Glutathionuria: gamma-glutamyl transpeptidase deficiency. *J Inherit Metab Dis.* 1980; 2(1):3–7. [PubMed: 6118466]
- Xiong Y, Uys JD, Tew KD, Townsend DM. S-glutathionylation: from molecular mechanisms to health outcomes. *Antioxid Redox Signal.* 2011; 15(1):233–270. [PubMed: 21235352]
- Xu K, Meng XY, Wu JW, Shen B, Shi YC, Wei Q. Diagnostic value of serum gamma-glutamyl transferase isoenzyme for hepatocellular carcinoma: a 10-year study. *Am J Gastroenterol.* 1992; 87(8):991–995. [PubMed: 1353662]
- Yamada K, Tsuji T, Kunieda T. Phenotypic characterization of Ggt1(dwg/dwg) mice, a mouse model for hereditary gamma-glutamyltransferase deficiency. *Exp Anim.* 2013; 62(2):151–157. [PubMed: 23615310]
- Yamamoto S, Watanabe B, Hiratake J, Tanaka R, Ohkita M, Matsumura Y. Preventive effect of GGsTop, a novel and selective gamma-glutamyl transpeptidase inhibitor, on ischemia/reperfusion-induced renal injury in rats. *J Pharmacol Exp Ther.* 2011; 339(3):945–951. [PubMed: 21937737]
- Yang L, Fox SA, Kirby JL, Troan BV, Hinton BT. Putative regulation of expression of members of the Ets variant 4 transcription factor family and their downstream targets in the rat epididymis. *Biol Reprod.* 2006; 74(4):714–720. [PubMed: 16394217]

- Yao DF, Huang ZW, Chen SZ, Huang JF, Lu JX, Xiao MB, et al. Diagnosis of hepatocellular carcinoma by quantitative detection of hepatoma-specific bands of serum gamma-glutamyltransferase. *Am J Clin Pathol.* 1998; 110(6):743–749. [PubMed: 9844586]
- Yoshikawa M, Tsuchihashi K, Ishimoto T, Yae T, Motohara T, Sugihara E, et al. xCT inhibition depletes CD44v-expressing tumor cells that are resistant to EGFR-targeted therapy in head and neck squamous cell carcinoma. *Cancer Res.* 2013; 73(6):1855–1866. [PubMed: 23319806]

Fig. 1.

The van der Waals surface of human GGT with the active site cleft facing the viewer. The large subunit (dark gray) and the small subunit (white) are shown with the catalytic Thr-381 (red) in the deepest part of the small subunit cleft. Four of the seven potential glycosylation sites are seen in this orientation, asparagine 110, 120, 230 and 344, and the basal *N*-acetyl glucosamine residue, which was identified in the crystal structure at each of these sites, is represented as dark orange van der Waals spheres. An anion-binding site (green) within the small subunit cleft is labeled 1103. This figure was originally published in *The Journal of Biological Chemistry*. West, M.B., Chen, Y., Wickham, S., Heroux, A., Cahill, K., Hanigan, M.H., Mooers, B.H.M., Novel insights into eukaryotic gamma-glutamyl transpeptidase 1

from the crystal structure of the glutamate bound human enzyme. *J. Biol. Chem.* 2013; 288(44):31902–31913. © the American Society for Biochemistry and Molecular Biology

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Fig. 2. Gamma-glutamyl bond. The structure of glutathione is shown glutamate (green) bound via a γ -glutamyl bond (arrow) to cysteine (red) and glycine (blue).

Fig. 3.

The Hydrolysis and Transpeptidation Reactions Catalyzed by GGT. (A) The physiological reaction catalyzed by GGT is the hydrolysis of gamma-glutamyl bonds. The hydrolysis reaction is shown with glutathione as the substrate, and shows the release of glutamate which is the product that is measured in the Glutamate Release Assay for GGT activity. (B) The transpeptidation reaction requires high concentration of dipeptide acceptor and is favored at pH 8 and higher. The transpeptidation reaction is shown with L-gamma-glutamyl para-nitroanalide (L-GpNA), the substrate used in the standard biochemical GGT assay, which monitors the release of pNA which is yellow. Adapted from a figure published in *Biochemical Journal*. Wickham, S., Regan, N., West, M. B., Thai, J., Cook, P. F., Terzyan, S. S., et al. (2013). Inhibition of human gamma-glutamyl transpeptidase: development of more potent, physiologically relevant, uncompetitive inhibitors. *Biochem J.* 2013; 450(3): 547–557@ The Biochemical Society.

Fig. 4.

Gamma-glutamyl transpeptidase (GGT)-positive tumors cleave extracellular reduced and oxidized glutathione (GSH and GSSG) providing an additional source of cysteine for intracellular GSH synthesis. GGT cleaves glutamate from GSH and GSSG. The cysteinylglycine dipeptides can be cleaved by any of several dipeptidases that are present on the surface of the cell. The glutamate, glycine, cystine and cysteine that are released from GSH and GSSG, are transported into the cell by the standard amino acid transporters. Cystine is taken up by the x_c⁻ cystine/glutamate antiporter (red). Cysteine is taken up by the ACS transporter (brown). Once inside the cell, cystine is reduced to cysteine by the strongly reducing environment of the cytoplasm. The first step in GSH synthesis is catalyzed by gamma-glutamyl cysteine synthetase (1) and the second step is catalyzed by GSH synthetase (2). Tumors are under redox stress which can be further increased by pro-oxidant therapy. GSH levels are depleted in tumors by several pathways. GSH is oxidized to GSSG as part of an ROS detoxification system that is present in both the cytoplasm and mitochondria. GSH peroxidases (3) catalyze the oxidation of GSH to GSSG. GSSG can be reduced to GSH by GSH reductase (4) and NADPH. However, under extreme oxidative stress GSSG is transported out of the cell by the multidrug resistance protein (MRP) transporters (blue). GSH is also depleted from the cell by binding to the electrophilic metabolites of chemotherapy drugs, which is catalyzed by GSH S-transferases (5). The GSH-conjugates are transported out of the cell by the MRP transporters (blue). Intracellular GSH can also be depleted by binding to intercellular proteins, a process known as protein glutathionylation.

Table I

Expression of GGT1 in Human Tumors

Tumor Type	#GGT+	# GGT–	% GGT-Positive
Hepatocellular Carcinoma *	9	0	100%
Renal Cell Carcinoma *	6	0	100%
Prostatic Adenocarcinoma *	70	1	99%
Pancreatic Adenocarcinoma *	10	1	91%
Invasive Breast Carcinoma *	60	21	74%
Ovarian Adenocarcinoma *	16	6	73%
Ovarian Adenocarcinoma **	33	12	73%

* Data from M. H. Hanigan, Frierson, Swanson, & De Young, 1999

** Data from M. H. Hanigan, Frierson, Brown, Lovell, & Taylor, 1994